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THE AMERICAN
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VOL. 130 JULY 1, 1940 No. 1

VASOCONSTRICTION IN RENAL HYPERTENSION ABOLISHED BY PITHING[1]

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With the assistance of BING MOY

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Received for publication November 6, 1939

Although no vasoconstrictor substance has been demonstrated in the blood of hypertensive patients, or in that of animals with experimental hypertension, most investigators of the problem incline to the belief that a peripherally active vasoconstrictor substance must be the actual cause of the increased arterial resistance (Goldblatt, 1940, summary of literature). Heymans and his colleagues, impressed with the rôle normally played by the carotid sinus and aortic reflexes, also believe that peripheral vasoconstriction and sensitization of vasomotor endings play a part, but insist that the reflex control mechanism must also be affected in order to have sustained arterial hypertension (Heymans, 1938).

Several years ago we reported (Dock and Rytand, 1934) that rats with renal hypertension had a prompt fall of pressure to the same level as controls when both groups had the central nervous system destroyed, but this evidence was obtained with indirect and unsatisfactory methods of measuring arterial pressure. That experiment has now been repeated, using rabbits and direct recording of arterial and venous pressure and the earlier observations have been confirmed. It is known that renal hypertension may occur after the sympathetic chains have been removed (Alpert et al., 1937; Freeman and Page, 1937; Heymans et al., 1937) or the cord below C4 destroyed (Glenn and Lasher, 1938; Glenn et al., 1938). Nevertheless the nervous system does play an important rôle in the mechanism of renal hypertension since pithing abolishes the difference between hypertensive and normal animals.

[1] This work was aided by a grant for medical research from the Rockefeller Foundation, and by the use of facilities of the Department of Pharmacology kindly offered by Professor Hanzlik.

METHODS. Normal rabbits or those which had been hypertensive for months due to a tie narrowing the renal artery were anesthetized, the lumbar cord exposed by laminectomy, and arterial pressure recorded continuously from a carotid artery. The cord was destroyed along with the dorsal part of the brain by pithing with a wire rod through the laminectomy opening, after tying both carotids and starting artificial respiration. Epinephrine, pitressin or renin were given intravenously through a jugular cannula and in nine animals the jugular venous pressure was observed in a manometer or recorded graphically. In two hypertensive and two control animals urethane anesthesia was used; the arterial pressure was low and the fall in relation to that in the central artery of the ear, measured indirectly before anesthesia, was much greater in the hypertensive than in normal animals. In three control and five hypertensive animals the splanchnic arterial bed was ligated before pithing. This prolonged the effect of epinephrine injections but did not prevent the fall of pressure due to pithing. The results to be reported were obtained with ether anesthesia and intact circulation, except for carotid ligation. In three hypertensive rabbits intravenous infusion of acacia-Locke's solution was used to raise venous pressure after pithing. Three hypertensive and two normal animals were pithed and the pressure allowed to fall to zero; in six hypertensives and fourteen normals repeated doses of epinephrine were given after pithing, in seven normals pithing was performed during continuous epinephrine infusion (two of these, which had many ectopic beats before pithing, went into ventricular fibrillation with immediate fall of pressure to zero on being pithed). In five normals pithing was performed at the height of a response to renin, generously supplied by Dr. I. H. Page of the Lilly Laboratories. In five hypertensive rabbits pithing was performed 30 hours after removal of the single kidney; all had normal levels of carotid pressure before being pithed.

RESULTS. In the normal rabbit there is no rise or a rise of less than 20 mm. Hg in arterial pressure during pithing. As pithing is completed there is an abrupt fall of pressure. This goes over into a more gradual fall at 30 to 50 mm., going steadily down or it may almost level off and decline gradually to zero. Prompt rise in pressure can be produced by epinephrine injection if the pressure has not fallen below 10 or 15 mm. Rabbits which have been hypertensive for months, but whose pressures fall to normal within 24 hours after total nephrectomy, react to pithing and to epinephrine in exactly the same way as normal animals.

When the arterial pressure of the normal rabbit is at a high level during continuous epinephrine infusion, there is no fall or only a transient fluctuation in arterial pressure on pithing; the pressure falls steadily to zero on discontinuing epinephrine. The effect of pithing rabbits at the height of response to renin was variable. In one, hypertension continued unchanged

for several minutes, in others there was a transient or a moderate but sustained fall, but the pressure always leveled off at a relatively high level, and declined very gradually as the effect of renin wore off. A second dose of renin, given to the pithed rabbit as the effect of the first dose wore off, again caused a rise. Thus renin, which with blood serum produces a vasoconstriction in isolated tissues of cats and dogs (Page and Helmer, 1940), has an effect somewhat like that of epinephrine. Its effect is very different from that of the humoral substance which causes hypertension of renal origin.

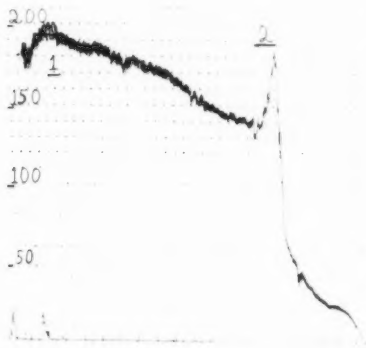

Fig. 1

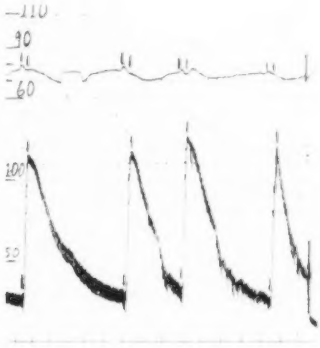

Fig. 2

Fig. 1. The initial rise in pressure, in this rabbit with renal hypertension, followed the starting of artificial respiration, *1*, and a fall occurred as the ether anesthesia became more profound. At *2* the animal was pithed and the pressure rose 40 mm. before its abrupt decline and more gradual fall to zero. Time record, 20 sec. intervals.

Fig. 2. Venous pressure (upper curve, in mm. H_2O) and arterial pressure (lower curve, mm. Hg) during four responses to epinephrine (15 gamma per kgm.) in a pithed normal rabbit. No rise in venous pressure precedes the rise in arterial pressure, save for the insignificant effect of the injection into the opposite jugular. It is quite obvious that the fall of arterial pressure is due to loss of arterial tone, not to low venous pressure. Time record 20 sec. intervals.

As contrasted with normal or recently nephrectomized hypertensive animals, rabbits with renal hypertension show more marked rise in pressure on ligating the second carotid artery, on starting artificial respiration, and during the destruction of the cord (figs. 1, 3). After the cord, medulla and pons of the hypertensive rabbits are destroyed by pithing, the pressure falls abruptly, reaching 20 mm. in one or two minutes. Occasionally, as in some normals, the pressure falls sharply to 30–50 mm., and then more slowly, reaching 20 mm. in 3 to 5 minutes. If epinephrine is given there is a sharp transient rise in pressure (fig. 3). Animals may be kept alive for several hours by repeated or continuous injections of epinephrine but they

show no recovery of tone; as soon as the epinephrine is stopped the pressure falls to zero. Ligation of the coeliac axis prior to pithing (3 hypertensives, 5 controls) modifies the result very little. It does prolong the effect of a dose of epinephrine, presumably by preventing its destruction in the liver. Raising the venous pressure by infusions of 10 to 15 cc. of Locke's solution or acacia-Locke's, per kilo of rabbit per minute for three to five minutes, does not suffice to maintain arterial pressure levels over 40 mm. Hg, although at the end of infusions, with arterial pressure steadily falling, venous pressure levels are 3 to 5 cm. H_2O above normal. On giving epine-

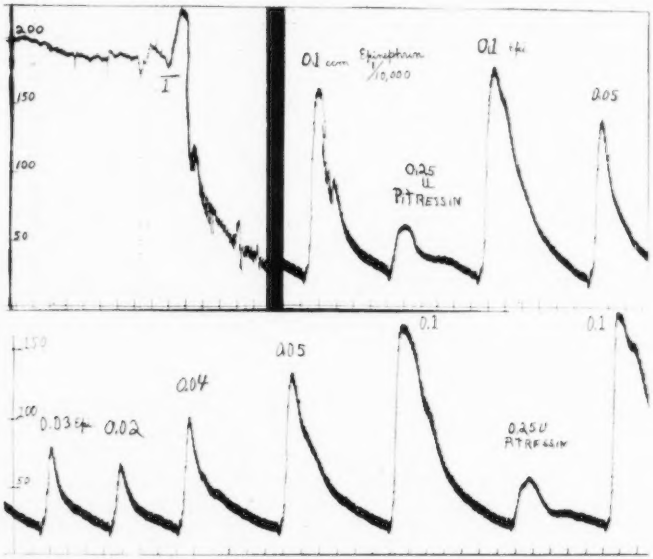

Fig. 3. Pressure in this hypertensive rabbit (3.8 kgm.) rose 35 mm. during pithing: abrupt and then gradual fall afterward. The maximal responses to epinephrine were obtained after 15 minutes. The first response to epinephrine was greater following each dose of pitressin. Time record, 20 sec. intervals.

phrine the pressure rises very sharply in the carotid, but the venous pressure either stays constant or with large doses stays at its original level for several seconds and rises after the arterial pressure has begun to fall from its peak (fig. 2). If the low arterial pressures were due only to inadequate venous return, no rise could occur following epinephrine until the venous pressure had risen markedly. Since this was not observed in any of the numerous cases in which venous pressure was noted, in hypertensive and control rabbits the fall in blood pressure must be ascribed to loss of arterial resistance rather than decrease in venous return after being pithed.

The response to epinephrine was more marked in the pithed hypertensive rabbits than in the controls or in hypertensive rabbits nephrectomized completely 30 hours before pithing. In order to minimize the effect of artificial respiration on the size of the response to epinephrine, varying doses were given repeatedly to an animal, and ventilation was increased until no further increase in response to each dose level was observed. It was always apparent that the maximal response to any given dose was

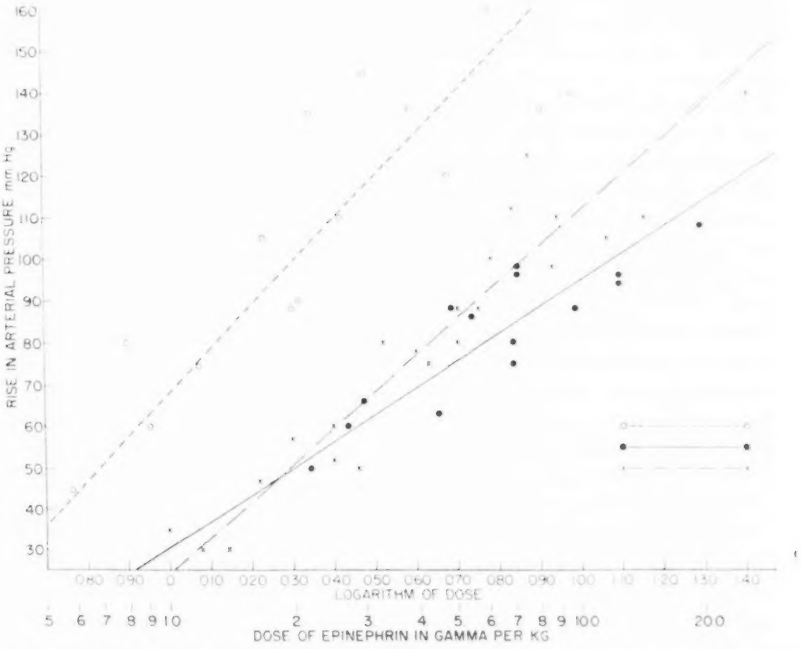

Fig. 4. Relation of rise in blood pressure, in pithed rabbits, to dose of epinephrine injected. Renal hypertensives, 0 and-----; normals, solid circles and line; renal hypertensives pithed 30 hours after nephrectomy, x and — .. Renal hypertensive rabbits lose their sensitivity to epinephrine after the kidney is removed and arterial pressure falls to normal.

much greater in rabbits which were hypertensive at the time they were pithed. The average sensitivity to epinephrine (k in the formula: rise in pressure $= k$ Log. of dose $+ a$), was 60 per cent greater in such rabbits than in normals or those nephrectomized 30 hours previously. Stated in another way it takes three times as much epinephrine to produce a rise of 50 mm. in the pithed normal rabbit as it does in pithed hypertensives and five times as much to produce a rise of 100 mm. In the three pairs of ani-

mals in which responses to pituitrin were noted, after pithing, the sensitivity of controls and hypertensives was the same. In unanesthetized intact rabbits with renal hypertension, pressor response to pituitrin is greater than in controls (Brown et al., 1939; Page and Ogden).

DISCUSSION. It can not be denied that pithing is likely to produce shock, but it does not interfere with response to any of the known peripherally active vasoconstrictors, and often is used to provide unusually sensitive preparations for demonstrating their action (Page and Helmer, 1940). In pithed rabbits given intravenous acacia or Locke's solution diuresis was marked, and it is difficult to see how a peripherally acting vasoconstrictor of renal origin could be inhibited even by a shocking procedure which does not diminish the effect of epinephrine, renin or pitressin, and does not inhibit renal function. On the other hand, study of the rôle of the sympathetic system or of the spinal cord can only be analyzed by means of such acute experiments, since the body possesses means for gradually reëstablishing the control of the central nervous system over the vascular bed when these normal pathways are destroyed (Brown and Maycock, 1940; Nowak and Walker, 1939). When the cord below C4 is destroyed, or the sympathetic system gradually ablated, normal blood pressure is restored and maintained. Renal hypertension less severe than in intact animals can then be produced, but since the mechanism by which normal pressure is maintained in animals with severed nerve tracts is unknown, such experiments cast little light on the mechanism of renal hypertension. The acute experiment does serve to show that the nervous system is as important in maintaining hypertension as it is in maintaining normal pressure levels.

Such observations suggest that an important part of the action of the humoral agent causing renal hypertension is to modify the "set" of the vasomotor mechanism, just as substances from inflamed or necrotic tissue modify the "set" of the heat regulating center. The same mechanisms are used to conserve heat or liberate extra heat in order to maintain the temperature at 40° in typhoid fever as are used to hold it at 37° under normal conditions. Since hypertensive animals and patients are able to regulate flow to the tissues, for temperature regulation or in response to exercise or nervous stimuli, in the same way as do normals, it seems not improbable that the normal regulatory mechanism continues to function, unaffected save for a change in the level at which the controlling center holds the mean arterial pressure.

At the time when the observations on sympathectomy or chronic cord damage in relation to renal hypertension were reported it was not realized that the nervous system could regain control of the vasomotor and cardioaccelerator mechanism after a lapse of days or weeks, and it was reasonable

to assume that the central nervous system and sympathetics were relatively unimportant in causation of renal hypertension. The occurrence of a fall in pressure when the spinal cord is severed in sympathectomized cats (Brown and Maycock, 1940) and of a gradual rise after section of the carotid and aortic nerves in sympathectomized dogs (Nowak and Walker, 1939) are examples of the persistence of central influences after section of the normal vasomotor pathways. The immediate but usually transient fall of pressure after splanchnic nerve section in hypertensive patients, and the acute experiments here described prove that the sympathetic and central nervous systems are important links in the mechanism causing chronic hypertension, renal or nonrenal in origin.

When it was observed in these experiments that pithed hypertensive animals are much more sensitive than pithed control rabbits, it was suspected that arterial hypertrophy might account for this, as the animals had been hypertensive for three to six months. For this reason sensitivity was studied after nephrectomy in animals which had been hypertensive for even longer periods, five to eight months. Under these conditions sensitivity fell to the normal level, as did mean carotid pressure, within 30 hours after removing the kidney which had caused the hypertension. This leaves no doubt that a substance which sensitizes to epinephrine and perhaps to vasomotor nerve stimuli is present in renal hypertension. Such a substance, acting only in this way, can scarcely cause hypertension, for if the moderator nerve reflexes were unaffected any rise in pressure would simply cut down the outflow of vasoconstrictor impulses, resulting in maintaining normal pressure with a lower rate of vasomotor tonic stimulation. Such a sensitization, along with an action on the vasomotor regulatory center raising the level at which tonic impulses were inhibited, would permit maintenance of hypertension without great increase in the rate of discharge from the center and ganglion cells along the vasomotor path.

SUMMARY

On pithing the central nervous system of rabbits those with renal hypertension have a rapid fall of blood pressure to as low a level as normal controls. Even holding venous pressure at high levels by intravenous infusion of acacia-Locke's solution fails to restore normal arterial pressure in these animals.

In pithed rabbits a rise in arterial pressure is easily evoked with epinephrine; the response is greater in those which have had renal hypertension for several months, but not in hypertensive rabbits nephrectomized 30 hours before pithing. No rise in venous pressure precedes the rise in arterial pressure. Renin, like epinephrine, causes a rise in arterial pressure in pithed rabbits, and thus differs from the humoral agent causing chronic

renal hypertension. The latter apparently changes the reaction of the vasomotor center so as to maintain pressure at high levels. It also increases the sensitivity of the arterial response to epinephrine.

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VARIABILITY OF BLOOD pH AND ITS ASSOCIATION WITH METEOROLOGICAL FACTORS

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The importance of the hydrogen ion concentration of the blood for a great variety of biological phenomena has long been recognized and observations on the normal human have revealed a range that is presumably rather narrow (1, 2, 3) though the reports are rather conflicting because in many instances seasonal factors have been neglected and also because of difference in technique. Variations that were associated with weather changes (barometric pressure) (4) as well as seasonal changes (5) have been reported by Petersen in humans. Studies on the influence of altitude and temperature on pH (7) have appeared from time to time, as well as observations extending over short periods. There has been a lack of controlled frequent observations on pH extending over prolonged periods.

Because of the difficulty of controlling "normal" human subjects over long periods of time, a study was first undertaken on the variations in pH over a period of a year in a group of "normal" dogs and compared with a series of simultaneous determinations made on groups of humans in order to determine whether any consistent similarities or differences were present.

EXPERIMENTAL. Employing a group of six dogs of approximately the same size and weight, blood was drawn from each dog in rotation on successive days in order to include in our determinations a picture of the daily variations that have been previously reported (4, 5), and to avoid the possibility of thrombosis and occlusion in the superficial veins which are likely to occur in a dog following daily venous punctures over prolonged periods. The animals were given a standard diet throughout the experiment.

The pH determinations were made on blood plasma according to the glass electrode method. The buffer solution was compared at weekly intervals with a standard pH solution. To assure constancy of our standard pH solutions they were checked every six weeks against numerous precision hydrogen electrode assemblies. The blood having been drawn under oil with a minimum of trauma and transferred under oil into constricted tubes, containing three drops of 20 per cent potassium oxalate, (the reaction of which was repeatedly tested throughout the experiment),

the determinations were made within fifteen minutes following all of the precautions recommended to insure accurate readings with our apparatus. The readings at room temperature were corrected after the method described by Myers and Muntwyler (6). Our readings were checked at intervals against readings made immediately following the withdrawal of

TABLE 1

Mean pH and standard errors for each dog

DOG	MEAN pH	STANDARD ERROR
1	7.43	±0.02
2	7.46	±0.02
3	7.47	±0.02
4	7.46	±0.02
6	7.47	±0.02
7	7.45	±0.02

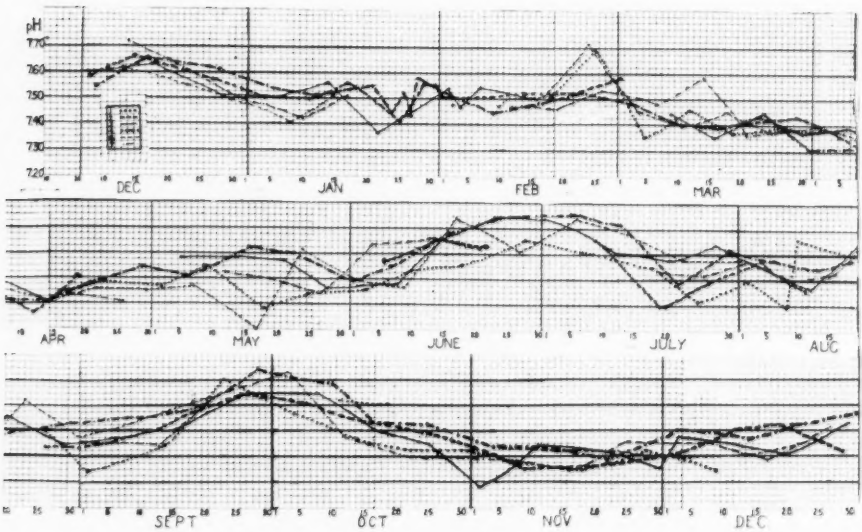

Fig. 1. A continuous record of pH determinations carried out on 6 dogs over a 13-month period. Each dog being taken in rotation on successive days of the week, and the weekly readings connected by a different line for each animal.

blood from the veins using a micro-chamber electrode. The Coleman pH electrometer was used which has a standard error of ± 0.01 pH.

After a period of training the pH determinations of the dogs were started at the beginning of December, 1937 and extended through December, 1938. The average pH and the standard errors were alike.

Figure 1 shows the character and the degree of fluctuations of the individual readings for each dog. The weekly readings for each dog were connected by a separate line; the relationships between the lines throughout the thirteen month period may be observed in the chart. In general, they present a similar pattern of variations throughout a relatively wide range. Some of the individual variations may be accounted for by the manner in which the readings were rotated from day to day. It has been pointed out (4, 5) that day by day determinations reveal a distinct variation of pH level associated with meteorological aberrations.

Groups of four to six humans were studied daily from January through December 1938. In general, the readings for each individual presented a similar pattern, rising and falling more or less synchronously.

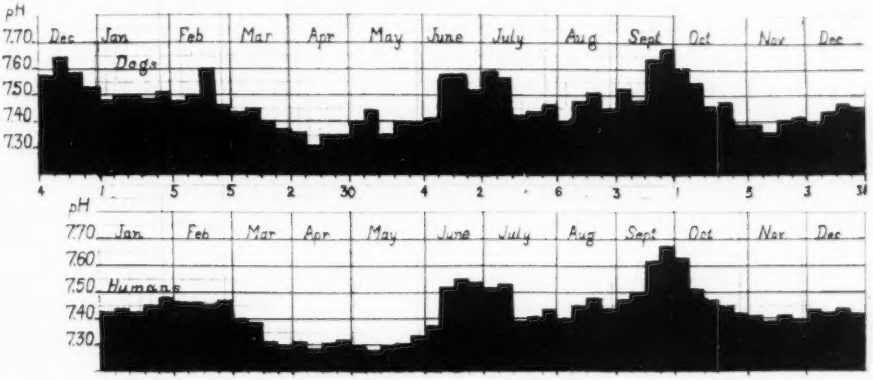

Fig. 2. Weekly average pH values for dogs (above) and humans (below).

Figure 2 shows the average pH level of the weekly cycle. The upper blocks of black represent the weekly average pH for the six dogs while the lower blocks represent the composite weekly average for the humans. Comparing the two series of blocks the close similarity may be seen, and statistically the correlation coefficient is high, being + 0.87.

From figures 1 and 2 it is evident that the pH of the various individuals (fig. 1) move in approximately the same manner. Further, the pH of two different species move in much the same fashion throughout the year (fig. 2). It is clear that at least a part of the variation of pH is determined, either directly or indirectly, by one or more factors to which all individuals studied (of either species) are exposed simultaneously. In endeavoring to determine what factors may account for these similar fluctuations in pH levels, a number of factors, both external and internal, were considered. Since the diets of the two species differed, the diet of the dogs being uniform through the year, the possibility that the fluctuations in pH might be

wholly due to seasonal differences in diet must be discarded. It was decided to investigate, if possible, the relationships between the several indices of the weather and the pH for both the humans and the dogs. Previous studies by Petersen (4) have given evidence of the association of the variations of a number of chemical constituents of the blood with the variations of barometric pressure and daily temperature.

The daily mean temperature, which was averaged to attain a single weekly figure, was obtained from the *Monthly Summary* of the U. S. Weather Bureau's Chicago Station. The daily 7 a.m. and 7 p.m. barometric pressure, which was likewise averaged to yield a weekly series, was obtained from the unpublished records of the Chicago Station.

The nature of the influence of the weather on pH, if it exists, is probably complicated and it may be necessary to start with certain simplifying assumptions regarding the possible functional relations. However, the nature of the functional relationship may be detected somewhat by an empirical study of the statistics. As each week has been considered as a discrete observation, some of the continuity has probably been lost in the pH series. Turning to the data itself, it has been observed that the pH was somewhat more stable than the barometric pressure, which at times showed great variability. For this reason it was decided to compute a three week moving average of the pH, barometric pressure, and temperature data.

By plotting pH against barometric pressure and temperature it was possible to judge the type of the functional relationship which exists between these variables. The charts clearly indicated that an arithmetical relationship was not involved, for the points tended to scatter in a fan-shaped manner. Conversion to logarithms seemed to reduce the scattering of the points in a manner which made their distribution relatively homoscedastic. Thus it appeared that it was a percentage change in pH which was associated with a percentage change in the barometric pressure. No definite conclusions could be reached concerning the relationship between pH and mean temperature and so the mean temperature was left on an arithmetic scale. In order to bring out most clearly the relationships, it was necessary to code the variables in the following manner: the 7.00 was dropped from the pH readings as the variation was in the last two figures, and then the reduced readings were multiplied by 4.00 to achieve a better transformation in logarithms. The barometric pressure was coded by subtracting 29.00 inches. For example, instead of 30.10 inches the figure of 1.10 inches was used. The mean temperature readings for the week were left untouched.

As the above codings were made prior to the transformation to logarithms, the various observations received somewhat different weights than those which a direct transformation would have given. The coding

also becomes a part of the description of the functional relationships between variables. The proposed mathematical function relating the pH to barometric pressure and mean temperature then becomes by the above adjustments:

$$\text{Log}\,[4.0(\text{pH}-7.00)] = \alpha + \beta \cdot \text{Log}\,[\text{Bar. P.}] + \delta\,[\text{Temp.}] \qquad \text{(Ia)}$$

or as an exponential:

$$\text{pH} = 7.00 + \frac{1}{4.0}[(10)^{\alpha}] \cdot [\text{Bar. P.}^{\beta}] \cdot [(10)^{\text{Temp.}\,\delta}] \qquad \text{(Ib)}$$

The statistics α, β and δ may be found by the method of least squares.

The Pearsonian product moment correlation coefficients for the coded variables were computed and the results between the pH readings, temperature and weather, are shown in table 2. The intercorrelation between the log of barometric pressure and mean temperature is -0.38.

Before answering the question which immediately suggests itself of whether these correlations are significantly different from zero, certain

TABLE 2

Zero order correlation coefficients

	BAROM. PRES.	TEMPERATURE
Human pH	+0.60	+0.18
Dog pH	+0.52	+0.19

reservations must be considered. In this instance we are not dealing with conditions of simple sampling for which the usual tests of significance were devised; the observations were not chosen at random, but are ordered in time; secondly, the data have been smoothed by means of the moving average, decreasing the true number of degrees of freedom available for determining the estimating equation.

A partial compensation for these difficulties may be gained by requiring a somewhat higher level of significance, say, the probability discarding a negative hypothesis (zero correlation) be set at 0.001 instead of 0.01. Further, it is necessary to consider the possibility that factors which could be neglected in the present study because they happened to remain relatively stable over the period studied might vary significantly in some later period. Turning to Fisher's table for testing of correlation coefficients (8), it is seen that if random samples of 50 paired observations each are taken from a universe of zero correlation, the correlation of ± 0.361 or greater is obtained only once in one hundred times. As the correlations with the barometric pressure for the pH of humans is $+0.60$ and for the pH of the dogs is $+0.52$, which are of greater value than the one per cent

level (± 0.361), even after considering the above reservations, it seems safe to conclude that the hypothesis that there is no correlation between pH and barometric pressure should be discarded as improbable.

The correlations with temperature and pH (dog, $+0.18$, and human, $+0.19$) are not to be considered significantly different from zero, as the 10 per cent level of Fisher's table indicated that a correlation of at least ± 0.230 or greater is obtained ten out of one hundred times with random sampling.

The intercorrelation between log of barometric pressure and temperature, of -0.3770, may be considered significantly different from zero. The probability of obtaining correlations which differ as much or more than $+0.60$ (human) and $+0.52$ (dog), if they are really drawn from the same universe, is 61 out of one hundred. It is necessary to conclude that there is no significant difference between the correlations.

Because of the importance of the intercorrelations between barometric pressure and mean temperature, it is clearly necessary to consider these two weather indices simultaneously. The parameters required in equations (Ia) and (Ib) are easily found and the estimating equations become: Human:

$$\text{Log}\,[4.0(\text{pH}-7.00)] = -0.1052 + 0.2022 \cdot \text{Log}\,[\text{Bar. P.}] + 0.002589\,[\text{Temp.}] \quad \text{(IIa)}$$

$$\text{pH} = 7.00 + 0.200 \cdot [\text{Bar. P.}]^{0.2022} \cdot [10]^{\text{Temp.}\,0.002589} \quad \text{(IIb)}$$

Dog:

$$\text{Log}\,[4.0(\text{pH}-7.00) = +0.0323 + 0.1379 \cdot \text{Log}\,[\text{Bar. P.}] + 0.001863\,[\text{Temp.}] \quad \text{(IIa}')$$

$$\text{pH} = 7.00 + 0.268\,[\text{Bar. P.}]^{0.1379} \cdot [10]^{\text{Temp.}\,0.001863} \quad \text{(IIb}')$$

The relative importance of the barometric pressure and temperature in determining the pH values is not well pictured by the above equations because the barometric pressure and temperature readings are measured in different units. If the variables are converted to units such that the standard deviations are equal, coefficients which are more comparable may be computed. Table 3 gives the *Betas*, or the coefficients in standard units. It is seen that the barometric pressure is of greater importance in the estimation of pH than is the temperature.

The accuracy with which the estimating equations (IIa) and (IIa′) predict the values of pH is given by the multiple correlation coefficient. The multiple correlation coefficient for the human pH with barometric pressure and temperature is 0.7383 and for the dog pH is 0.6670. Both of these correlations are significantly different from zero.

The importance of the interplay of barometric pressure and temperature is clearly brought out when we turn to the partial correlations. A partial correlation attempts to give a picture of the effect of one of the independent variables—say, barometric pressure—upon the dependent variable, pH, when the other independent variable or variables, say, temperature, are held constant. It is the statistical approximation to the control of certain important influencing factors in the experiment conducted in a laboratory.

The partial correlations as compared to the zero order correlations (table 2) have in all instances been increased due to the negative correlation between the temperature and barometric pressure. While each of the weather indices tends to affect the pH in a positive fashion, they tend to move in opposite directions resulting in a partial negation of the net effects upon pH.

TABLE 3

Coefficients of estimating equations in standard units

	BAROM. PRES.	TEMPERATURE
Human pH	+0.77	+0.47
Dog pH	+0.69	+0.45

TABLE 4

Partial correlation coefficients

	BAROM. PRES.	TEMPERATURE
Human pH	+0.73	+0.54
Dog pH	+0.65	+0.49

All four partial correlations in table 4 can be considered as significantly different from zero as a partial correlation of ± 0.37 or greater is obtained only one per cent of the time from a universe of zero correlation (Fisher's table 5, A (8)). The use of partial correlational technique clearly brings out the importance of considering both temperature and barometric pressure and it is seen that temperature does play an important part in the determination of the pH.

Until now it has been assumed that the relationships between the variables as adjusted by transformation into logarithms were necessarily rectilinear; however, it may be well to investigate the possibility of curvelinearity. The method of successive approximation suggested by Ezekial (9) was used. The residuals from the estimating equations are plotted against: first, the barometric pressure; and secondly, against the temperature, correcting the regression if the straight line is not visually the "best fit." If these new regressions still do not satisfy, adjustments are made

again or until the best curvelinear regressions are found. The disadvantage of the method, of course is that in the end no mathematical equation is obtained unless the work of fitting is repeated, assuming another general

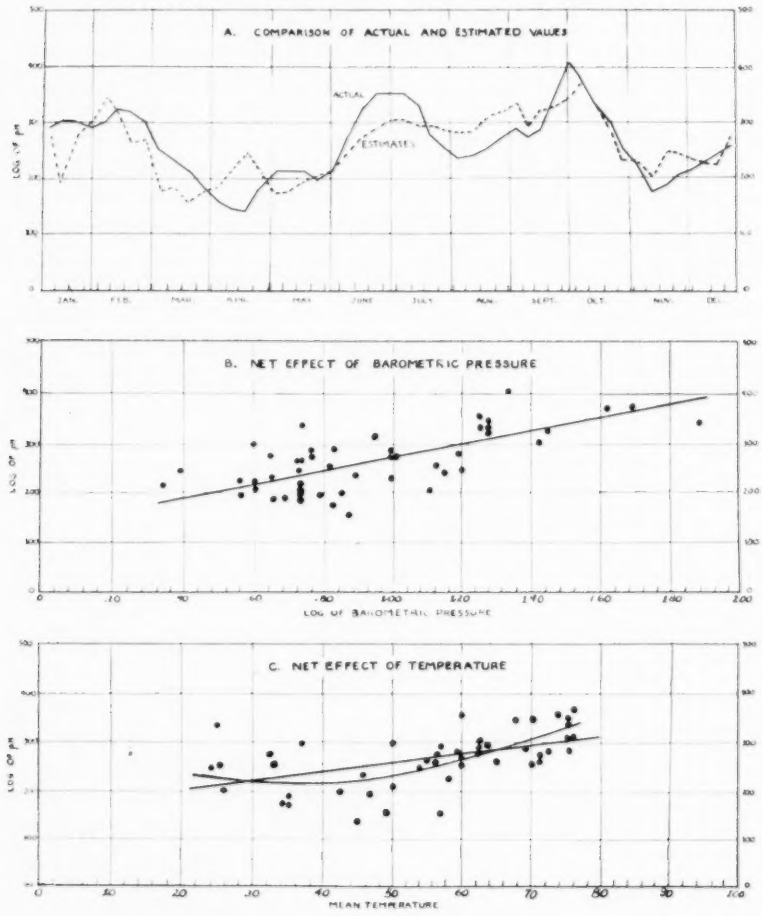

Fig. 3. pH of dogs: section A, comparison of logarithms of actual values and logarithms of pH as estimated from barometric pressure and mean temperature; section B, the net effect of barometric pressure on pH; section C, the net effect of mean temperature on pH.

equation which seems to meet the requirements of the corrections for curvelinearity. Further, there is no adequate way to determine the number of degrees of freedom absorbed by the correction.

In figure 3 are shown the corrections for curvelinearity for the dog pH. Section B shows the net effect of the barometric pressure and clearly indicates that the influence is linear. The lowest section of the figure shows that the assumption of linearity does not hold. As temperature rises from a weekly average of 42° the pH rises at an increasing rate. Below a temperature of 42° the pH seems to rise slightly. The nature of the relationship may be due to the living conditions of the animals, as they were kept in steam-heated buildings and were not exposed to the extreme cold, while during the summer they were not as well protected against excessive heat. It may be that during the coldest days they were living under much warmer conditions than during only moderately cold days, or the possibility of compensating physiological mechanisms must be considered. In section A the actual values of the logarithms of pH as observed are compared to the values estimated from the relations shown in the lower sections of the chart. It is seen that the estimates follow the actual values quite closely. The importance of curvelinearity of the temperature relationship is reflected in the increase of the linear multiple correlation coefficient of 0.6670 to the curvelinear index of multiple correlation of 0.7345.

Figure 4, in turn, shows the similar material for the human pH. The relationship of the barometric pressure and pH is slightly curvelinear as is shown in section B. The pH level rises more rapidly as the barometer moves from lower level than when the increases occur at the higher levels. The deviation from linearity may not be significant and it may be that no real difference exists between the reaction of the pH of the dogs and the pH of the humans to barometric pressure. In section C the relationship of the pH of the humans with temperature shows the same curvelinear tendencies as were discovered for the dogs. A low pH is reached at about 42° with pH increasing more rapidly as temperature rises; slight increases in pH occur with drops in temperature below 42°. The latter phenomenon might also be attributed to our ability to protect ourselves against the cold on the one hand by clothes and warm indoor temperatures, or on the other hand, by a possible compensatory mechanism. In section A will be found the comparison of the actual and estimated values, the correspondence of which is somewhat closer than for the dogs. The importance of the curvelinearity is evidenced by the increase in the linear multiple correlation of 0.7383 to a curvelinear Index of Multiple Correlation of 0.8224. This is an important increase in the "closeness of fit," for the standard error of estimate has dropped from 0.45 to 0.25, or only 25 per cent of the variance of the logarithms of pH is left unexplained as compared to 45 per cent under the assumption of linearity.

Discussion. Because of the high correlation between dogs and humans even with the relatively wide fluctuations in pH—from 7.32 to 7.68—

some of the complex mechanisms involving the pH of each may be briefly considered together. The pH measured was that of venous blood of an

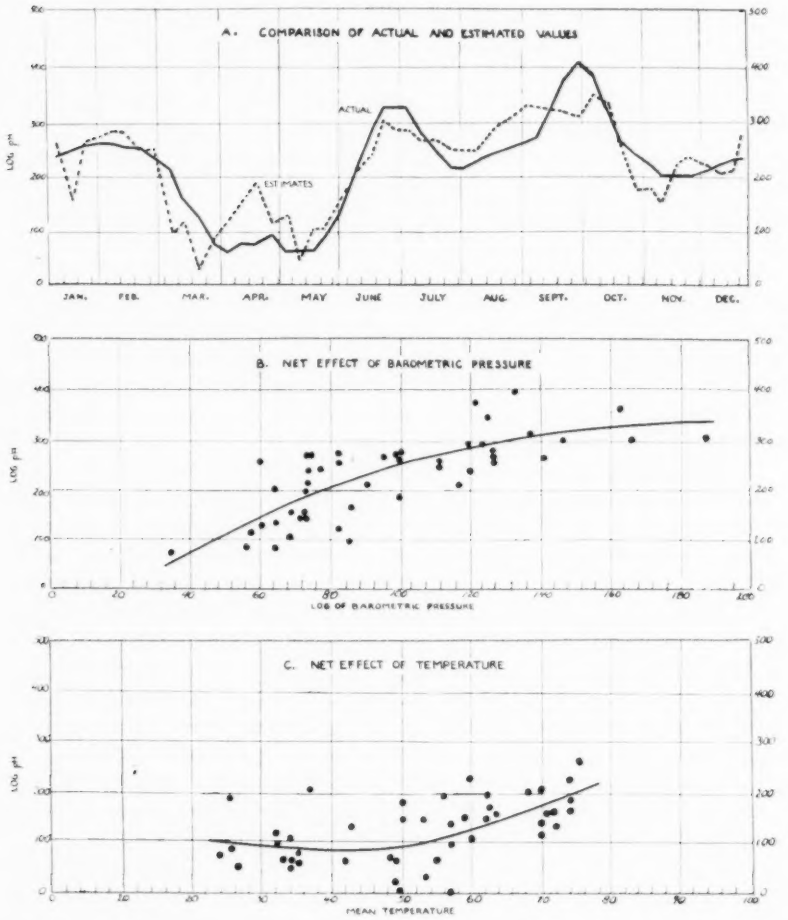

Fig. 4. pH of humans: section A, comparison of logarithms of actual values and logarithms of pH as estimated from barometric pressure and mean temperature; section B, the net effect of barometric pressure on pH; section C, the net effect of mean temperature on pH.

extremity and thus was possibly subject to wider variations than arterial blood. Since pH is a measure of the hydrogen ion activity (10) in a biologic system at a given time, variations in the buffers (ratio of base to

bicarbonate), the rate of oxidation and reduction, and the rate of elimination of metabolic products will affect the pH. In comparing the CO_2 contents of the blood with the pH a general reciprocal relation was seen. However, the CO_2 content was subject to wide fluctuations without corresponding changes in the pH. The CO_2 content depends upon (7) the amount of available alkali (alkali reserve) and the amount of hemoglobin which, like other acids, competes for the alkali, as well as upon the ratio of production to elimination. Increasing the environmental temperature lowers the CO_2 content (7) and inversely tends to raise the pH. Although temperature and barometric pressure are acting simultaneously (and the importance of their interplay has been demonstrated above), for purposes of simplification and clarity it may be well to discuss them separately.

It was seen that the pH levels tended to rise at an increasing rate as the temperature rises above 42°, however, with the temperature falling below 42° the pH seems to rise slightly. While the temperature has a definite effect on the pH, the effect of temperature was found to be not as great as barometric pressure which may be due to the ability of the organism to compensate readily for changes such as variation in the CO_2 content, for example, through the respiratory mechanism. Furthermore, as barometric pressure and temperature tend to move in opposite directions, some of the effects of temperature appear to be partially negated. However, at the higher levels of temperature which are associated with an increased respiratory rate, more CO_2 is blown off and the pH rises at an increasing rate.

Changes in barometric pressure are statistically definitely more important than changes in temperature. The relationship appears to be a linear one with a leveling off of pH at the high pressure readings. The distinctly higher correlation between the barometric pressure and pH is probably due to a more complex mechanism perhaps involving such factors as circulatory changes (5), the oxygen tension of the blood (increasing pressure increases oxygen content) and the compensatory effects at various levels and the greater release of acid metabolites associated with anoxia. Changes in pH in turn, have been shown to influence the oxidation rates of various organs and tissues with increasing rates at lower pH (11, 12, 13, 14).

Suggestive among the factors which may possibly influence the pH are variations in the energy production. Petersen (5) has observed changes in B.M.R. and pH, associated with meteorological alterations. Ritzmann and Benedict (15) have shown variations in B.M.R. which they believed might be due to seasonal changes in the weather. Correspondingly distinct seasonal variations in the organic iodine content in the thyroid of domestic animals has been recently reported by Seidell and Fenger (16).

With abrupt changes in temperature and barometric pressure (polar fronts), the importance of circulatory phenomena (vascular spasm and relaxation) have been brought out (5), and these have been associated with fluctuations in the blood chemistry, among them pH (rising and falling pH). When meteorological stimuli were either very pronounced or frequent, summation effects were observed. Statistically significant daily variations in pH have also been reported by Shock and Hastings (3) and the data showed general trends over a period of several days.

Considerable controversy has arisen in discussing seasonal influences, due to failures to duplicate results. That this must occur is logical, for one spring may not be like another spring and one May not like another May. The temperature may be low and the barometric pressure high, or they may be unstable one year and more stable the next. One cannot compare corresponding months of different years and consider them similar only because of their order in time. On such a basis failures to duplicate results cannot be considered as discounting climatic influence. In comparing pH values collected in previous years (5) in this laboratory with those presented in this paper, definite differences were encountered. In a number of years, for example, the levels in the spring were low—in some there was no decline—while in others they were relatively high.

SUMMARY

1. A high correlation was found in the pattern and in the degree of variation of the pH of the blood among a group of dogs studied over a period of one year.

2. A high statistical correlation was obtained in comparing the continuous pH values in a group of dogs with those of a group of humans for a period of one year.

3. A relatively wide degree of fluctuation was observed in both humans and dogs. In man the weekly averages varied from 7.28 to 7.68; while in the dogs they varied from 7.32 to 7.68.

4. In comparing the pH and the weather, statistically significant coefficients were found with barometric pressure and temperature, with a distinctly higher correlation being found for barometric pressure than for temperature. Increases in pH were associated with increases in barometric pressure. On the other hand, the relationship between pH and temperature was not as marked and only at higher temperatures were increases in pH definitely associated with increases in temperature. In attempting to evaluate seasonal influences varying results were found, depending upon the weather indices discussed.

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DEMONSTRATION OF THE LIBERATION OF RENIN INTO THE BLOOD STREAM FROM KIDNEYS OF ANIMALS MADE HYPERTENSIVE BY CELLOPHANE PERINEPHRITIS

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It has been shown that the perinephric reaction to cellophane and silk produces a constricting hull around the parenchyma of the kidneys which causes persistent arterial hypertension (1). The renin-angiotonin vasopressor system is believed to be involved in the genesis of this type of experimental hypertension. In short, the results of studies on this system have shown that renin is not itself a pressor substance because extensive purification yields an enzyme-like substance without vasoconstrictor action in organs perfused with Ringer's solution (2). Fractionation of blood produces a substance (renin-activator) which when added to the purified renin caused the reaction mixture to assume powerful vasoconstrictor properties (3). The product of the interaction of renin and renin-activator was isolated, crystallized and named angiotonin (4, 5). Angiotonin also has an activator separable from blood (6). The kidneys form inhibitors to both renin and angiotonin (7).

With these observations in mind, it was obviously important to demonstrate that the kidneys of hypertensive animals did in fact liberate renin and in amounts greater than from normal ones. Employing normal kidneys perfused with blood, Kohlstaedt and Page (8) found that reduction of pulse pressure led to the liberation of a substance from the renal vein which reacted with purified renin-activator to produce strong vasoconstriction. Since the reaction between renin and renin-activator appears to be specific, there was good reason to believe that angiotonin had been produced by the interaction of renin liberated from the perfused kidney and renin-activator added to the blood from the renal vein. Kidneys perfused at normal pulse pressure liberated only small amounts or no renin.

We have now studied the blood from normal kidneys and kidneys with their parenchyma constricted by the fibro-collagenous hull incited by cellophane or silk perinephritis. Since it has been shown that injection of renin quickly exhausts the supply of renin-activator present in normal blood (7), it would be anticipated that, if renin were liberated in large

amounts from the kidneys, blood from the renal vein would contain little renin-activator and excess renin. To demonstrate this, renin-activator was added to the plasma, the mixture incubated, and tested for angiotonin by means of the perfused rabbit's ear preparation.

METHODS. Hypertension was produced by application of cellophane or silk to the parenchyma of the kidneys of normal and uninephrectomized dogs. In some animals the kidneys were transplanted up under the skin by the method of Page and Corcoran (9) to facilitate drawing of renal vein blood.

After severe hypertension was established, renal venous and femoral arterial and venous blood samples were drawn, heparinized, and, after centrifuging, the plasma separated. The renin content of the plasma was ascertained by circulating a mixture of plasma with renin-activator, prepared by the method of Kohlstaedt, Page, and Helmer (10), through a rabbit's ear perfused by pulsatile pressure with Ringer-Locke's solution. Usually 0.2 cc. of plasma was mixed with activator in the proportion of 1 to 17, incubated at 25°C. for 10 minutes and then injected into the perfusing fluid. The plasma was also tested for its angiotonin- and renin-activator content by addition respectively of angiotonin and renin.

RESULTS. What we believe to be examples of typical experiments are given in table 1.

Addition of renin-activator to plasma from the renal vein of normal dogs causes only moderate constriction, when the same amount of plasma or renin-activator alone causes none. But plasma from the renal vein of a hypertensive dog, provided the hypertension is not malignant, on addition of activator causes 2 to 6 times as much constriction as normal. There is no great difference between femoral arterial and venous blood though often femoral blood appears to contain slightly more renin. Neither contain amounts of renin comparable with that in the renal vein.

Addition of renin to plasma in the proportion of 1 part to 17 parts of plasma, produced a mixture with marked constrictor properties, the renal vein blood usually being somewhat less active than femoral venous or arterial blood. The femoral arterial blood from hypertensive dogs produced greater constriction than femoral blood from normal dogs.

Addition of angiotonin to plasma caused marked vasoconstriction regardless of the origin of the blood, but femoral arterial blood from hypertensive animals gave distinctly greater than normal responses, with femoral venous blood next in order of potency.

It is of especial interest that, in 3 dogs with the syndrome of malignant hypertension, addition of renin-activator to plasma from the renal vein caused little vasoconstriction, nor did addition of renin or angiotonin. Large amounts of renin were found in the renal vein of one dog early in the course of malignant hypertension.

TABLE 1

The effect of addition of renin-activator, renin, and angiotonin on the vasoconstrictor properties of plasma from renal and other blood vessels when perfused through an isolated rabbit's ear

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
			Reduction of flow through ear vessels						
	mm. Hg		*minutes*	*per cent*	*minutes*	*per cent*	*minutes*	*per cent*	
1	126	R. R. V.	½	20	1¼	54	3	62	Both kidneys explanted. No anesthetic
		F. V.	¼	20	1½	60	3	70	
		F. A.	¼	23	2	63	4	64	
1	170	L. R. V.	7	82	½	12	½	20	Both kidneys explanted. No anesthesia. Left renal artery constricted 10 days before
		R. R. V.	½	20	1	38	1	28	
		F. V.	1	49	1	50	1	32	
		F. A.	¾	31	1½	44	1½	39	
1	178	L. R. V.	5½	84	4	47	4	51	Same 3 days later
		R. R. V.	3	49	7	65	4	46	
		F. V.	1½	47	10	81	10	90	
		F. A.	1½	40	10	76	12	77	
1	182	L. R. V.	8	94					Same 4 days later
		R. R. V.	½	28					
1	166	L. R. V.	3	72	1	47			Same 5 days later and after right nephrectomy
		F. V.	½	18	2½	56			
		F. A.			3	64			
1	166	L. R. V.	3	81	1	16	1	32	7 days after right nephrectomy
		F. V.	2	72	1	60	3	59	
		F. A.	½	21	2½	79	5	65	
1	180	L. R. V.	3	57			½	17	13 days after nephrectomy. Signs of malignant hypertension. B.U.N. = 56 mgm.
		F. V.	1½	63			½	26	
		F. A.	½	22			1	47	
1	189	L. R. V.	2½	57			¾	24	14 days after nephrectomy. Anuria
		F. V.	½	26			½	26	
		F. A.	0	0			1	39	
1	192	L. R. V.	2½	42	¾	34	½	25	15 days after nephrectomy. Just after loosening clamp. B.U.N. = 133 mgm. Died 2 days later
		F. A.	½	10	1¼	39	1¾	51	

TABLE 1—*Continued*

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
			minutes	*per cent*	*minutes*	*per cent*	*minutes*	*per cent*	
Malignant hypertension									
2	182	R. R. V.	8	94	½	33			Lt. nephrectomy + Rt. silk
		F. A.	2	46	1	39			
		Inferior vena cava 3 cm. above renal veins	5	68	1	43			Malignant hypertension
		Inferior vena cava above hepatic veins	3	61		43			
3	126	L. R. V.	2½	54					Malignant hypertension. Rt. nephrectomy + silk on Lt. kidney. B.U.N. = 32.0. Retinal detachment and hemorrhages obser. 2 days before. 4 days before B.P. = 210 mm. Hg
		F. V.	¼	14					
		F. A.	½	17					
4	140	L. R. V.	1½	30	1	31	1	36	Right + left silk. Malignant hypertension
		R. R. V.	1	29	2½	54	1½	57	
		F. A.	0	0	2	47	1½	43	
5	142	F. A.			1½	20	1	12	Malignant hypertension
6	202	F. A.			½	20	¼	29	Malignant hypertension. B.U.N. = 21.5 mgm.
7	224	F. A.					½	34	Malignant hypertension. B.U.N. = 75.0 mgm.
8	88	F. A.			½	40	1½	44	Both kidneys in cellophane. Malignant hypertension. B.U.N. = 90.8 mgm.
Normal									
9		F. A.			3	69	2	46	Normal dog
10		F. A.			2	40	1	37	Normal dog
11		F. A.			2	30	1	37	Normal dog
					1	40	1	41	

TABLE 1—*Concluded*

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
			minutes	*per cent*	*minutes*	*per cent*	*minutes*	*per cent*	
Normal—*Continued*									
12	*mm. Hg* 120	L. R. V.	1	68	¾	26	2½	57	Normal dog under pentobarbital anesthesia
		R. R. V.	½	30	1½	55			
		F. V.	¼	46	1½	39	3	61	
		F. A.	½	18	2	43	3	64	
13	130	L. R. V.	¼	11	1	41	1¼	47	Normal dog under pentobarbital anesthesia
		F. V.	¾	19	1½	58	1	68	
		F. A.	¾	15	1½	61	1	52	
14	240	L. R. V.	½	31	2	70			Normal dog under pentobarbital anesthesia
		R. R. V.	¼	40					
		F. V.	½	39	2	65			
		F. A.	½	48	2	78			
Renal hypertension									
15	182	L. R. V.	2¾	79	½	18	1¼	54	Both kidneys in cellophane. Anesthetized with pentobarbital
		F. V.	½	20	2	47	4	79	
		F. A.	½	22	5	72	3½	71	
16	180	L. R. V.	1¼	46	2	70			Both kidneys in cellophane
		F. V.	1	40	2	72			
		F. A.	1	42	2½	75	2½	58	
17	182	L. R. V.	5	69	1	29	1	41	Rt. nephrectomy and Lt. kidney in silk
		F. V.	1½	32	1	32	1½	47	
		F. A.	1	34	3	47	2	48	
18	200	L. R. V.			½	21			Rt. nephrectomy and Lt. kidney in silk. Under pentobarbital anesthesia
		F. V.			2½	48			
		F. A.			5½	79			
19	172	R. R. V.	3½	78	1½	61	2	47	Rt. kidney explanted Lt. nephrectomy. Rt. renal artery constricted 3 days before
		R. R. A.	1	46					
		F. V.	1½	49	1½	57	3	51	
		F. A.	1	54	4½	74	4	68	
19	170	R. V.	8	94	3	64			Same 7 days after constriction
		F. V.	2½	47	2½	59			

* L. R. V. = left renal vein, R. R. V. = right renal vein, F. V. = femoral vein, F. A. = femoral artery.

Discussion. From these experiments it appears that the demonstration of renin in blood depends on the presence of sufficient renin-activator with which it can react to form angiotonin. If this is true, it is understandable why many attempts to show that renin is liberated into the renal vein blood have failed.

When renin is injected in large amounts into animals, at least two reactions are provoked and either one or both may lead to neutralization of the pressor action of the renin (7). The first of these is exhaustion of renin-activator which is demonstrable in perfused isolated organs. The second is liberation of an inhibitor or anti-pressor substance from the kidneys and muscle. In normal animals the amount of inhibitor to both renin and angiotonin appears to be high, in the hypertensive animal much less, and in the nephrectomized animal it is almost lacking (6). This suggests that heightened susceptibility to angiotonin may be a vital circumstance for the development of persistent arterial hypertension.

When normal kidneys are perfused at normal pulse pressure, little or no renin is demonstrable in the renal vein blood (8) but if the pulse pressure is reduced, large amounts are liberated as shown by the fact that addition of renin-activator causes marked vasoconstriction when the mixture of renal vein plasma and activator are perfused through a rabbit's ear. We believe that liberation of renin in increased amounts has now been demonstrated from kidneys of animals made hypertensive by perinephritis, and in two experiments, by constricting the renal artery (Goldblatt). The amount of renin appears to diminish rapidly in its course through the circulation and by the time the blood has reached the femoral artery it contains at most only slightly increased amounts. This may be explained on the basis of the reaction of renin with renin-activator to produce angiotonin (4, 5).

The amount of renin-activator in femoral arterial and venous blood apparently increases in hypertension (10) and is slightly decreased in renal vein blood. This may be due either to an actual increase in production of activator, to decrease in the amount of inhibition, or both. Therefore, until it is possible to distinguish between increased amounts of activator and decreased amounts of inhibitor, no exact description can be given.

Angiotonin-activator is present in the blood of both normal and hypertensive animals regardless of source, but greater than normal amounts may be found in femoral arterial and venous blood of hypertensive animals.

The animals exhibiting the syndrome of malignant hypertension appear to be characterized by the fact that addition of neither renin nor angiotonin to plasma from the femoral vessels causes marked vasoconstriction. Since addition of activator does not greatly augment the vasoconstrictor action of the plasma, it may be surmised that the inhibitor content of the blood is elevated. In one animal (no. 2) relatively early in the course of the disease, addition of renin-activator to plasma from the renal vein caused

marked vasoconstriction, indicating liberation of renin. The plasma from the renal veins from 3 others (nos. 1, 3, 4) exhibited little more renin than normal. Femoral arterial blood was low normal or distinctly subnormal in its angiotonin- and renin-activator content.

CONCLUSIONS

1. Renin is liberated into the renal vein in increased amounts by the kidneys of dogs made hypertensive by cellophane or silk perinephritis, and by clamping the renal artery. Most of it disappears by the time the blood has reached the femoral artery.

2. Renin-activator is decreased in the blood from the renal vein and is increased in hypertensive animals when the femoral artery is reached.

3. Angiotonin-activator is not greatly decreased in the renal vein blood but may be increased in the femoral arterial blood in hypertensive animals.

4. Early in the course of malignant hypertension, large amounts of renin are liberated by the kidneys (1 experiment). Later, both angiotonin-activator and renin-activator are greatly reduced or sufficient inhibitor is formed to abolish the reaction between them and angiotonin or renin (7 experiments).

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DIFFERENCE IN THE ACTIVATING EFFECT OF NORMAL AND HYPERTENSIVE PLASMA ON INTESTINAL SEGMENTS TREATED WITH RENIN

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It has been shown by Kohlstaedt, Helmer and Page (1938) that renin itself is not a pressor substance but when it reacts with an activator contained in blood, prompt vasoconstrictor properties are manifest. This is due, as shown by Page and Helmer (1939), to formation of a heat-stable substance which they have called angiotonin and from which crystalline derivatives have been prepared.

With the hope that some relatively simple biological test object could be found for semi-quantitative assay of the amount of activator in blood, studies were conducted on intestinal segments suspended in Ringer's solution. It is with these data that the present communication is concerned.

METHOD. Rabbits were killed by a blow on the neck, a piece of intestine removed 15 cm. from the pylorus, and after gentle cleaning away of intestinal contents, rings 2.5 cm. long cut. These were suspended in baths of oxygenated Ringer's solution[1] at 37°C. The baths were equipped with automatic temperature regulators and were of 40 cc. capacity. The renin employed was prepared by the method of Helmer and Page (1939). Plasma was prepared from heparinized blood and used within an hour after the blood was drawn. In several experiments no difference was observed in fresh plasma and plasma which had stood 24 hours in the refrigerator.

The experiments were conducted as follows. After the intestinal segment was contracting rhythmically and steadily, 1 or 2 cc. samples of

[1] Composition of Ringer's solution:

	grams per liter
Sodium chloride	9.0
Potassium chloride	0.417
Calcium chloride	0.24
Magnesium chloride	0.062
Sodium bicarbonate	4.0
Dextrose	8.0

plasma were added to the bath to insure that the plasma itself contained no constrictor substance. The segment was then washed twice with Ringer's solution and 2 cc. of renin added and allowed to remain for 5 minutes. After washing the segment three times, 2 cc. of plasma were again added and the constrictor action recorded. Without further treatment with renin, plasma was repeatedly tested until it was certain that the response was relatively constant. Segments of the same intestine were tested simultaneously with normal and hypertensive blood in the two baths of the apparatus.

In a few experiments, angiotonin prepared by the method of Page and Helmer (1940) was used.

Reaction of intestine to various constituents of the renin-angiotonin vasopressor system. Renin-activator prepared by fractional precipitation of

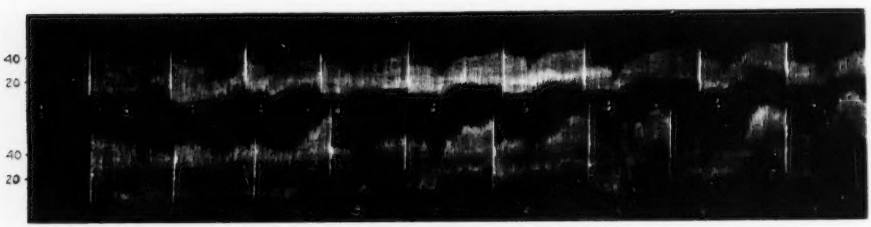

Fig. 1. Contractor action of plasma from normo- and hypertensive patients on isolated rabbit's intestine after treatment with renin. Number 1, 3 plasma (2 cc.) from patient with normal arterial pressure (114, 70 mm. Hg); 2, 4 plasma (2 cc.) from hypertensive patient (188, 118 mm. Hg); 5, 6 renin (2 cc.) in contact with intestine for 7 minutes; 7, 9, 11, 13, 15, 17, 19 addition of normal plasma; 8, 10, 12, 14, 16, 18 addition of hypertensive plasma.

blood with potassium phosphate when added to the bath in amounts of 1 to 2 cc. caused no contraction of the intestine. Neither ultra-filtrates of renin or of renin-activator were active either alone or mixed. Boiled renin plus boiled renin-activator was also ineffective. But if renin (0.1 cc.) and renin-activator (1.7 cc.) were mixed and added, immediate contraction occurred. The quantitative aspects of this reaction have been dealt with more fully by Page (1939).

Plasma (2 cc.) usually causes no contraction and renin (2 cc.) may or may not do so. Since plasma contains renin-activator, mixing renin and plasma yields a contractor substance. The contractor substance is doubtless angiotonin for angiotonin itself causes marked and sharp contraction of the intestine when added to the bath. Furthermore, repeated doses of angiotonin sensitize the intestine markedly, but treatment of the intestine with angiotonin does not cause it to respond when plasma is added subsequently.

These reactions have been employed as a basis for roughly estimating the amount of renin-activator in plasma. Renin is added to the tissue and then the excess washed away. When plasma containing activator is added, angiotonin is formed which causes the intestine to contract. It

TABLE 1

Contraction of intestine as a result of treatment with renin followed by plasma from normal and hypertensive persons

SUBJECT	B.P.	CONTRACTION IN MILLIMETERS OF INTESTINE AFTER ADDING SUCCESSIVE DOSES OF PLASMA						
		1	2	3	4	5	6	
Human hypertension								
	mm. Hg							
Normal	126/90	2	0	0	0	0	0	1 cc. plasma
Hypertensive	188/100	15	14	15	15	17	20	
Normal	120/70	0	0	0	0	0		
Hypertensive	170/90	20	9	8	10	12		
Normal	128/68	0	2	2	4	2	2	
Hypertensive	200/136	0	6	9	12	9	8	
Normal	120/70	1	2	1	2	1		
Hypertensive	198/118	36	9	13	14	20		
Normal	130/80	4	0	0	0	0	0	
Hypertensive	200/136	14	8	2	13	6	26	
Normal	115/70	4	4	6	5	13	12	2 cc. plasma
Hypertensive	198/118	12	19	19	27	30	38	
Normal	124/84	5	5	3	6	5	6	
Hypertensive	210/140	11	20	23	26	24	23	
Experimental hypertension								
	MEAN B.P.							
Normal	128	3	0	3	0	2	3	1 cc. plasma
Hypertensive	187	16	15	18	20	19	23	
Normal	130	0	0	0	0	0		
Hypertensive	180	7	3	10	10	10		
Normal	135	4	4	3	3	5		3 cc. plasma
Hypertensive	190	28	20	19	21	32		
Normal	130	5	5	3				
Hypertensive	186	12	10	14				
Normal	122	0	8	8	5			
Hypertensive	170	1	14	13	12			

probably also sensitizes it to further angiotonin formation. Hence, when the first plasma is washed away and a second, third or fourth dose of plasma is added, without re-treatment of the tissue with renin, contraction occurs.

Assay of plasma of normal and hypertensive persons and dogs. The bloods assayed were drawn from patients with typical essential hypertension and

from dogs with hypertension produced by the perinephritis method of Page (1939).

In plasma of both hypertensive persons and dogs, there appears to be greater activating power for renin than in normal blood (table 1).

Discussion. Intestinal segments react with contraction to many substances or to changes in the conditions of the experiment. It is for this reason important to keep the conditions constant and to demonstrate that the reaction being tested is a specific one. These conditions have, we believe, been fulfilled in these experiments.

One other circumstance must, however, be kept in mind in interpreting these results. Page and Helmer (1940) were able to demonstrate the occurrence of an inhibitor to renin and angiotonin in blood of normal animals. It is therefore possible that instead of renin-activator being increased in hypertension, the amount of inhibitor is reduced. At present there is no way to decide between the alternatives, or the third one, that both may occur. Since nephrectomy appears to abolish one chief source of inhibitor, it is possible that lack of inhibitor causes increased activating power of hypertensive plasma.

It is probable that, under the experimental conditions described, renin is adsorbed by the tissue and, when renin-activator is added as contained in plasma, interaction occurs with liberation of angiotonin. Angiotonin causes the intestine to contract. Being water-soluble and easily diffusible, it is dissipated when the tissue is washed but enough renin is retained in the tissue to react with the next dose of renin-activator. Since angiotonin sensitizes the tissue, it is able to respond to smaller amounts of it. Addition of angiotonin itself is unable to produce the phenomenon because it is washed away with each washing of the tissue.

Plasma from certain hypertensive persons and from dogs with cellophane perinephritis and hypertension exhibits greater than normal power to activate renin. This adds one more bit of evidence suggesting that the humoral mechanism responsible for essential hypertension in human beings is similar to that in dogs with experimental hypertension. It also confirms, by an entirely different method, Kohlstaedt, Page and Helmer's (1940) observation that the renin-activating power of plasma is increased in experimental hypertension.

CONCLUSIONS

1. The reaction between renin and renin-activator is a specific one and the product of this reaction—angiotonin—causes strong contraction of isolated intestinal segments. This reaction has been employed to ascertain the renin-activating power of plasma.

2. Heparinized plasma derived from blood of some patients with essential hypertension causes greater renin-activation than does normal human

blood. Plasma from dogs with experimental hypertension also exhibits this heightened power compared with plasma of normal dogs. This suggests that the humoral mechanism in the two types of hypertension have much in common and that the hypertensive either has increased amounts of renin-activator in the blood, or decreased amounts of renin-inhibitor, or both.

I wish to express my appreciation to Joseph L. Haug for technical assistance.

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THE EFFECTS OF HYPERTHERMIA AND HYPOTHERMIA ON CERVICAL LYMPH FLOW[1]

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The experiments on the effects of local heat and cold upon cervical lymph flow (McCarrell, 1939–1940) suggested a further study of lymph formation and movement as a result of general heating and cooling of the body. The recent therapeutic use of hyperthermia and hypothermia has stimulated interest in the physiological changes, particularly those involving the circulatory system, brought about by abnormally high or low body temperatures. That alterations in lymph flow probably occur as a result of these circulatory changes has often been inferred, but actual experimental proof is meager. Ōtsuka (1936) reports an increase in thoracic duct flow in dogs after the injection of typhoid vaccine or "thermin." The highest temperature recorded was 41.1°C., and the lymph flow reached its maximum and fell off before the height of the fever was attained. No literature has been found on the subject of lymph flow during hypothermia.

The present study deals with the changes in lymph flow and protein content that occur in anesthetized dogs and cats subjected to high or to low environmental temperatures under such conditions as to cause profound body temperature alterations. The lowest body temperature obtained was 25°C., the highest 45°C. The experiments were acute, and thus no attempt has been made to determine the effects of prolonged exposure to extreme environmental temperatures.

METHOD. The experiments were carried out in an air-conditioned room in which temperature and humidity could be controlled at will. The animals were anesthetized by an intraperitoneal injection of nembutal (40 mgm. per kgm.). The right and left cervical lymphatics and the thoracic duct were cannulated at the base of the neck. The snout of the animal was attached by rubber bands to three metal uprights at the head of the animal board and by a double length of twine to a motor driven crank. As the crank revolved, the head was slowly flexed and extended, and thus a constant flow of cervical lymph was produced. The lymph, removed

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from the cannulae during definite periods of time (10 or 15 min.), was collected in weighed tubes. The tubes were reweighed and the lymph flow calculated in grams per minute (thoracic duct) or in milligrams per minute (cervical). Protein percentage was determined refractometrically in all cervical lymph samples and in a few thoracic duct samples. The fat content of the latter was generally so high that accurate refractometric readings were impossible. The details of the above procedures have been described previously (McCarrell, 1939).

Before beginning lymph collections, the animal was given 300 to 500 cc. of warm saline solution by stomach tube to assure an adequate supply of water. Body temperature was recorded by a mercury thermometer, the bulb being inserted into the abdominal cavity through a small slit in the abdominal wall and secured with a purse string suture. In the majority of the experiments, subcutaneous temperatures of the head were obtained with a mercury thermometer sewed in place under the skin of the left cheek. Venous pressure was measured at intervals in the right external jugular vein by means of a hypodermic needle attached to a saline-filled manometer. Mean arterial blood pressure was recorded from the femoral artery with a mercury manometer, and pulse pressure with a membrane manometer. An intravenous injection of 1 cc. of a 1 per cent solution of curare was given at the beginning of each experiment to prevent alterations in lymph flow due to panting or shivering. Artificial respiration was administered through a tracheal cannula.

Each animal was prepared and a preliminary control period carried out at a room temperature of 25 to 28°C. For the heat experiments, the room temperature was then raised as quickly as possible to 40 or 45°C., 30 to 35 minutes being necessary to accomplish this. The high external temperature was maintained for periods ranging from $1\frac{1}{2}$ to $3\frac{1}{2}$ hours, depending upon the type of experiment. In some cases the room temperature was reduced to the control level as soon as significant changes in lymph flow were observed; in others, the heating was continued and the body temperature allowed to rise until circulatory collapse developed and the animal died. The average relative humidity of the room air was 11 per cent.

For the cold experiments the room temperature was reduced to about 10°C. by drawing cold outside air through the ventilating system. The animals were subjected to this cool environment for periods ranging from 2 to $3\frac{1}{2}$ hours. Curare prevented shivering and the body temperature, which decreased gradually, reached minimum values of 25.6 to 29.7°C. before the experiments were terminated.

Nine dogs and two cats were studied under conditions of hyperthermia, and three dogs during hypothermia.

Results. 1. *Heat.* In these artificially respirated animals, panting was abolished by curare and the mouth was tightly closed with the twine

attaching the snout to the revolving crank. Thus the evaporation of moisture from the surfaces of the mouth and respiratory passages—the principal method of heat dissipation in dogs and cats—was reduced to a minimum. Under these conditions the body temperature began to rise soon after the room temperature was increased. The rise continued as long as the environmental temperature remained high, and, unless checked, led to circulatory collapse and the death of the animal.

The control period values for thoracic duct lymph flow in dogs ranged from 0.198 to 1.486 grams per minute; in one cat it was 0.134 gram per

TABLE 1

Cervical lymph flow and protein per cent during progressive hyperthermia

NUMBER OF ANIMAL	CONTROL PERIOD			FIRST CRITICAL TEMPERATURE			SECOND CRITICAL TEMPERATURE		
	Temperature	Average lymph flow	Protein	Temperature	Average lymph flow	Protein	Temperature	Maximum lymph flow	Protein
				Dogs					
	°C.	mgm./min.	per cent	°C.	mgm./min.	per cent	°C.	mgm./min.	per cent
1	37.0	28.3	3.46	41.1	50.2	2.86			
2	35.0	13.8	4.97	39.1	61.3	2.89			
3	38.5	16.8	5.22	39.9	72.8	4.78			
4	38.0	29.6	2.56	40.5	42.7	2.02	42.0	95.8	1.57
5	35.8	21.5	5.23	38.3	39.1	4.82	41.9	325.0	2.97
6	36.9	54.7	4.56	38.8	84.3	4.03	43.3	252.5	2.32
7	37.8	15.1	3.58	38.5	37.0	2.97	42.8	79.9	1.92
8	36.8	8.1	4.01	38.6	15.8	3.82	42.4	148.0	2.43
9	36.8	4.8	3.85	38.4	13.2	3.42	43.0	Saline injection before maximum flow reached	
				Cats					
10	37.0	23.8	4.02	None			43.4	60.7	2.75
11	37.0	3.9	4.18	40.0	14.2	3.58	43.5	30.9	2.04

minute. The flow tended to remain constant throughout the heating period up to the time of collapse, although in some experiments wide fluctuations occurred. A change was sometimes noted just before the death of the animal, but the direction of the change was not consistent. No general statement can be made as to the effect of hyperthermia on thoracic duct flow or protein content.

The cervical lymph flow, on the other hand, showed clear-cut changes at two separate critical levels of body temperature. Table 1 summarizes results obtained from the individual experiments. As the body tempera-

ture increased, cervical lymph production remained the same or less than the control period values until the first critical temperature zone was reached. At this point the flow began to increase, and attained values that were 1.4 to 4.5 times the original amounts. This increase was first noticed in dogs at body temperatures ranging from 38.3 to 41.1°C. Subcutaneous temperatures of the head were 0.3 to 0.6°C. higher than the corresponding temperatures in the abdominal cavity. The protein percentage decreased slightly as the lymph flow became more abundant. Reducing the body temperature restored the lymph flow to control values. One of the two cats failed to show a first critical temperature, the lymph flow remaining at the control level until collapse occurred. The second cat reacted similarly to the dogs, with a definite increase in cervical flow at 39.8°C.

If the body temperature continued to rise, the cervical flow was maintained at this higher level until a second critical temperature zone was reached at 41.9 to 43.5°C. At this point a tremendous increase in the cervical flow occurred, with ultimate values 3 to 18 times the control amounts being obtained. This augmented flow was characterized by a marked decrease in the percentage of lymph protein, the percentage at the height of the flow being approximately one-half that of the control period. The lymph flow fell off sharply just before death. The latter occurred in both dogs and cats when the body temperature reached 45.3 to 45.7°C.

Certain circulatory changes were brought about by the rising body temperature. An initial temporary decrease of mean arterial blood pressure occurred in some of the animals at the onset of the heating period, and was in most cases accompanied by a transitory decrease in pulse pressure. As the heating period continued, the mean arterial pressure and the pulse pressure in the majority of the animals showed gradual increases. At the time of the first lymph flow increase, the arterial pressure rise amounted to 6 to 35 mm. of mercury; that of the pulse pressure, 4 to 23 mm. of mercury. The arterial pressure in one dog failed to change, and in another dog exhibited a gradual decrease which continued throughout the experiment. The pulse pressure increased in all the dogs, decreased in one cat, and showed no change in the other cat. No correlation could be found between the relative amount of cervical lymph production and the relative pressure changes. No significant alterations of venous pressure were evident during this early period.

The circulatory changes occurring at the time of the second critical temperature were spectacular and quite uniform throughout the entire experimental series. The mean arterial pressure, which had remained the same or increased slightly above the level attained at the first lymph flow increase, exhibited a marked progressive fall at the time of the second tremendously augmented flow. The pulse pressure, likewise, began to

decrease at this time. The venous pressure increased 2.2 to 5.4 cm. of salt solution above the previous levels and then fell off rapidly as the circulatory collapse, which eventually caused the death of the animal, progressed.

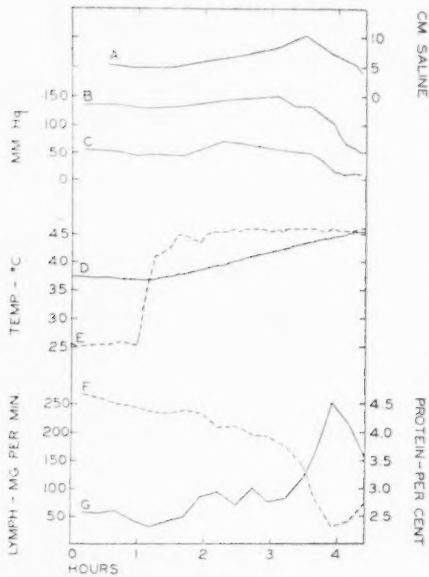

Fig. 1

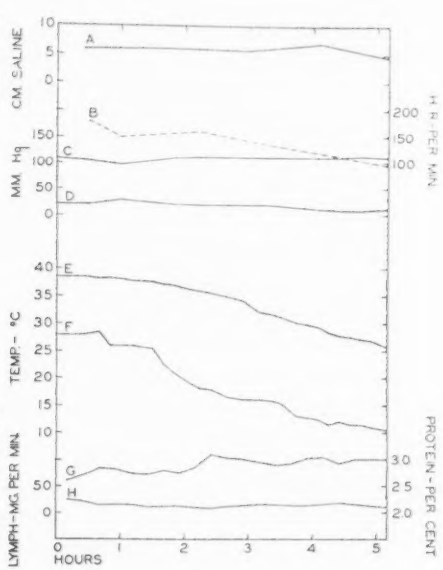

Fig. 2

Fig. 1. Circulatory and cervical lymph data during progressive hyperthermia. *A*, venous pressure in centimeters of saline; *B*, mean arterial blood pressure in millimeters of mercury; *C*, pulse pressure in millimeters of mercury; *D*, body temperature in degrees Centigrade; *E*, room temperature in degrees Centigrade; *F*, cervical lymph protein per cent; *G*, cervical lymph flow in milligrams per minute. First critical temperature 38.8°C.; second critical temperature 43.3°C. See text.

Fig. 2. Circulatory and cervical lymph data during progressive hypothermia. *A*, venous pressure in centimeters of saline; *B*, heart rate per minute; *C*, mean arterial blood pressure in millimeters of mercury; *D*, pulse pressure in millimeters of mercury; *E*, body temperature in degrees Centigrade; *F*, room temperature in degrees Centigrade; *G*, cervical lymph protein per cent; *H*, cervical lymph flow in milligrams per minute.

Figure 1, from data obtained during experiment number 6, shows the typical circulatory and cervical lymph changes which took place as the body temperature was gradually raised to a lethal level.

2. *Cold.* No striking changes were noticed in the cervical lymph flow as the body temperature decreased from approximately 38°C. to a minimum of 25.6°C. Subcutaneous temperatures throughout the cooling

period were 1 to 3 degrees lower than the corresponding abdominal cavity temperatures. There was a tendency for the flow to become slightly less and the protein percentage slightly higher than the control period values, but this situation is not uncommon when lymph is collected over a period of several hours. No conclusions could be drawn with regard to thoracic duct flow.

The circulatory manifestations, with the exception of the heart rate, were surprisingly inconspicuous, considering the magnitude of the body temperature change. The heart rate began to decrease at a body temperature of 35 to 36°C. and the rate became progressively slower as the hypothermia continued. Mean arterial pressure, pulse pressure, and venous pressure exhibited no consistent or significant alteration. Figure 2 represents the results of one typical experiment.

Discussion. It is generally agreed that dilatation of peripheral blood vessels is a fundamental physiological reaction to an increased environmental temperature. Bazett (1938a) lists the factors which may cause this dilatation as: 1, the direct effect of heat on the vessels; 2, a reflex inhibition of vasomotor tone due to stimulation of surface receptors, and 3, inhibition of vasomotor tone through a temperature rise in the hypothalamic region. Vasoconstriction, with absorption of fluid in the splanchnic area, in addition to contraction of the spleen are compensatory mechanisms brought into action at the same time in an attempt to maintain an adequate blood supply to essential organs (Bazett, 1938b).

The rise in cervical lymph flow occurring at the first critical temperature (38.3 to 41.1°C.) I believe is due to an accelerated rate of capillary filtration. This is brought about, first, by arteriolar dilatation which increases the capillary pressure, and secondly, by capillary dilatation which enlarges the area available for filtration. Landis (1934) reports these as normal responses in a heated area. This shift of capillary fluid which leads to a greater flow of more dilute lymph, often at a body temperature only slightly above normal, may explain the heat edema sometimes noticed in the tropics and may be a factor in the common summer experience of swollen hands and feet.

The circulatory collapse exhibited in the present experiments was similar to that described for dogs subjected to excessive moist heat (Hartman and Major, 1935) and for humans who have collapsed, sometimes fatally, during or after hyperpyrexia treatments (Kopp and Soloman, 1937). Kopp and Soloman consider this condition an example of a typical shock syndrome. There is such marked dilatation of capillaries, arterioles, and veins that the normal compensatory mechanisms are overshadowed and an extremely serious condition develops. An inadequate venous return leading to stasis and anoxemia increases capillary permeability and causes the loss of an abnormal amount of fluid from the blood stream. Kopp and Solo-

man state that collapse occurred in their human cases at a body temperature of 106°F. (41.1°C.) or above. This is somewhat lower than the minimum collapse temperature for dogs and cats. However, circulatory distress in humans undergoing hyperthermia treatment can be augmented by uncompensated fluid loss through skin and lungs. Gibson and Kopp (1938) find this loss may range from 6 to 32 per cent of the plasma volume. Reduction of blood volume in this manner was not an important factor in the experiments under discussion. Collapse in these dogs and cats was caused by a reduction in the effective blood volume due, first, to the loss of fluid with the increase in capillary permeability, and secondly, to the uncompensated vascular dilatation.

In two instances, attempts were made to relieve the symptoms of circulatory collapse by reducing the body temperature and at the same time administering large amounts of fluid intravenously. In one experiment the body temperature had reached 43°C., arterial pressure had dropped to 85 mm. of mercury, and cervical lymph flow was showing a characteristic increase. The room temperature was then reduced to 25°C., and with the aid of wet cloths and a fan the body temperature was lowered to 38°C. During this time, 400 cc. of Ringer's solution were given intravenously. The experiment was terminated too soon to determine lasting effects, but a fair circulatory recovery was obtained and the animal seemed in good condition. In a similar experiment with a higher body temperature (44.8°C.) and a lower arterial pressure (65 mm. Hg) recovery was incomplete even with the intravenous injection of 600 cc. of a 6 per cent acacia solution. This illustrates the rather narrow margins concerned when dealing with a condition as serious as heat shock.

The tremendously increased lymph flow which appeared in the early stages of collapse must have been due, first, to temporary venous pressure rise which would tend to increase capillary pressure, and secondly, to an increased capillary permeability caused by stasis and anoxemia. The volume of lymph was so great that a protein percentage increase, usually indicative of definite capillary damage, was not apparent.

The findings of Yannett and Darrow (1938) indicate a further possible source of fluid which might eventually contribute to lymph production. They found that a shift of water from brain cells to extracellular fluid occurred in cats with rectal temperatures 4 to 6°C. above normal. There were no significant changes in liver or muscle and no determinations were done on subcutaneous tissue.

The first reaction to exposure to cold is vasoconstriction of peripheral vessels. This reduces blood flow and, therefore, the loss of heat from the surface of the body. If the skin temperature becomes low enough (0 to 10°C.), capillary dilatation and hyperemia, as described by Lewis (1927), may occur. Apparently, capillary conditions, even at a minimum body

temperature of 25°C., are not sufficiently altered to cause changes in the amount of capillary fluid transudation that can be detected in the cervical lymph flow.

Hamilton and Barbour (1925) placed dogs on blocks of ice, and report that the muscle and subcutaneous tissues of the cooled side had a greater water content than the uncooled side. Landis (1934) remarks that the difference may have been due to gravity. No temperatures were given, and it is possible that the skin temperature may have become sufficiently low to cause capillary dilatation and hyperemia. Hamilton, Dresbach and Hamilton (1937) subjected rats and kittens to severe hypothermia, and, with the exception of a slowed heart rate and loss of spontaneous movements, found no marked physiological changes at body temperatures as low as 75°F. (23.9°C.). At 65°F. (18.3°C.) or less, they report a marked subcutaneous edema with profound disturbances of the nervous, vascular, and respiratory systems. The edema shown by these animals was undoubtedly an expression of the circulatory disintegration that occurs as a lethal temperature is approached. Jackson and Alonge (1934) found that 62 per cent of a large group of rabbits exposed to extreme cold died when the body temperature fell to 19°C.

In the new hypothermia treatment for cancer (Smith and Fay, 1939), with rectal temperatures reduced to 85 or 90°F. (29 to 32°C.), it is probable that the lymphatic circulation may be reduced to some extent, but it is believed that any effects of the low body temperature in this respect are not pronounced.

SUMMARY

Nine dogs and two cats, anesthetized and curarized, were subjected to a high environmental temperature (40 to 45°C.) at an average relative humidity of 11 per cent. The hyperthermia thus produced caused marked circulatory changes culminating in circulatory collapse and death at 45.3 to 45.7°C. Cervical lymph flow increased and protein percentage decreased at two critical body temperature levels. The first rise, which amounted to 1.4 to 4.5 times the control values and which occurred at a body temperature of 38.3 to 41.1°C., was due to an increase in the rate of capillary filtration caused by peripheral hyperemia. The second increase in cervical lymph flow, amounting to 3 to 18 times the normal, appeared at a temperature of 41.9 to 43.5°C., was coincident with the beginning of circulatory collapse, and was caused by a tremendous increase in capillary filtration resulting from a high venous pressure and from capillary stasis and anoxemia leading to injury to the capillary endothelium.

Cervical lymph flow in three dogs subjected to low environmental temperatures (10°C. minimum) failed to be significantly altered when the body temperature was reduced to a minimum value of 25.6°C. With the

exception of a decreased heart rate, circulatory changes were not pronounced at this degree of hypothermia.

Thoracic duct flow tended to be so variable that no conclusions can be drawn as to the possible influence of changes in body temperature on its production.

The writer wishes to thank Dr. Cecil K. Drinker for suggesting this problem and for his advice during its execution. Miss Hope King's assistance is also much appreciated.

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THE FLOW, PRESSURE, AND COMPOSITION OF CARDIAC LYMPH

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THE LYMPHATIC SUPPLY OF THE HEART. In a recent anatomical paper, Patek (1939) has summarized the literature upon the cardiac lymphatic vessels, and then has added very thorough observations made by modern injection methods in the normally beating heart of the dog. Patek's findings were as follows:

1. There is a plexus of large and small lymphatic capillaries under the epicardium, the larger vessels lying practically upon the muscle and receiving afferents from the myocardial lymphatic plexus. The subepicardial vessels empty into drainage trunks which accompany the coronary blood vessels. *These drainage trunks eventually unite into a single trunk which drains the entire heart and leaves it on the anterior surface of the pulmonary artery.* Valves occur in the draining lymphatics, but are rare in the subepicardial lymphatic capillaries.

2. In the myocardium there is an extensive plexus of lymphatics, the smallest being about three times the diameter of the blood capillaries in the same region. There are no collecting trunks in the myocardium, and all the lymph in these vessels passes into the large subepicardial lymphatic capillaries on the outer surface of the heart muscle. The myocardial lymphatic plexus is very uniform in density from the subendocardial to the subepicardial vessels, and valves are rare.

3. The third plexus of lymphatic capillaries is subendocardial and drains into the myocardial plexus. Valves are uncommon.

In summary, lymph formed in any part of the beating heart passes to the subepicardial plexus and then into the valved draining trunks. The movements of the heart must force lymph in the three systems of lymphatic capillaries in various directions, but since they are all connected and since there is but a single way for lymph to leave the heart, there is apparently steady drainage into the large valved trunks which accompany the coronary vessels and unite into the final single cardiac lymphatic.

In our experiments, we have cannulated this lymphatic after it emerges

from the pericardium and before it enters a very constantly placed lymph node shown in figure 1. We believe that the lymph collected by means of this cannula represents the complete lymph flow from the heart and from no other structures. Our reasons for this belief are as follows:

a. Alterations in flow and composition of lymph taken from the cannula follow immediately upon changes in cardiac activity.

b. When dyes are injected in the heart, we have found them in this lymphatic alone. Furthermore, with a cannula in the cardiac lymphatic

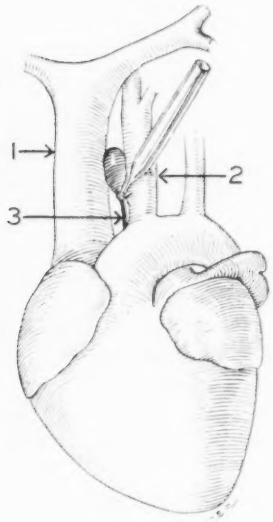

Fig. 1

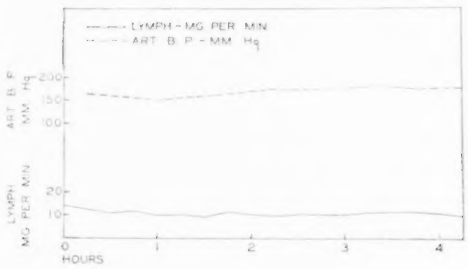

Fig. 2

Fig. 1. Diagrammatic sketch of the anterior surface of the dog's heart and certain of the great vessels. *1*, superior vena cava; *2*, innominate artery; *3*, cardiac lymphatic cannulated just prior to entering the cardiac lymph node.

Fig. 2. Normal flow of cardiac lymph. *Ordinates*, milligrams of lymph per minute and arterial blood pressure; *abscissae*, time in hours.

so that connection between this vessel and the thoracic duct is broken, no dye appears in either the thoracic or right lymphatic ducts after such injection into the heart muscle.

c. We have considered that lymph from the lungs might be mixed with the cardiac lymph, but have been unable to devise acute injection experiments which would prove this point. But on a single occasion an experimental dog showed pronounced pulmonary anthracosis, and lymph nodes at the root of the lung were quite black, being filled with pigment

carried to them by the lung lymphatics. On careful histological examination with the polarizing microscope, there were many fine unphagocytosed particles of crystalline silica in the pulmonary node and none in the cardiac node. It may be said that all particles were filtered out in the pulmonary node and that clear lymph from the lungs reached the heart node, but in the case of finely divided crystalline silica this does not happen. The material is not taken wholly effectually by phagocytes, and where two nodes are in series the second invariably receives a quota; and since these highly refractile particles are so easily recognized by the polarizing microscope, conclusions as to their absence are entirely safe and we believe this accidental finding, which must have followed a long period of dust inhalation, is important evidence that the cardiac lymphatic we have cannulated carries lymph from the heart alone.

EXPERIMENTAL TECHNIQUE. Dogs of above 10 kgm. weight are desirable, but in all work upon the lymphatics it soon becomes evident to the experimenter that the size of lymphatics and the degree of lymph flow are not wholly dependent upon size of animal. Anesthesia is accomplished by intravenous injection of nembutal, repeated in small amounts as required.

After tracheal cannulation and establishment of artificial respiration, the thorax is opened by removal of a section of the first three ribs about three or four centimeters from their sternal connections. The mammary arteries are then tied and the sternum sawed through just below the third rib, a block tie being passed around the lower end of the sternum to prevent venous oozing. This operation exposes the base of the heart and the great vessels. If any considerable amount of thymus tissue is present it should be removed, but usually the thymus does not extend as low as the site of cannulation of the cardiac lymphatic. This vessel is located by means of the cardiac lymph node, and its discovery will be greatly facilitated if bleeding into the loose tissues of the mediastinum has been thoroughly checked so that the operative field is clean.

In the beginning of our experience we found the vessel by the injection of a blue dye, T-1824, into the cardiac muscle, making a small hole in the pericardium and injecting into the muscle of the right ventricle by means of a fine needle. This injection will be necessary for any one who begins to look for this lymphatic, since it is embedded in fat and loose connective tissue, and, uninjected, is not easily seen save through the light of experience plus low-power binocular magnification. The vessel is usually about of the diameter of an ordinary pin. It is cleaned and tied, and then swells somewhat, which facilitates the incision for cannulation. The fine cannulae used are made of pyrex glass, 4 mm. in outside diameter and from neck to end are 7.5 cm. in length. The movements of the heart

and great vessels are always disturbing, but if the cardiac lymphatic is freed sufficiently cannulation can usually be accomplished, and the length of the cannula permits fastening it loosely in place by a stay suture in the left edge of the thoracic wound. The tip of the cannula in the cardiac lymphatic often rests upon the innominate artery and moves continuously during the several hours of the experiment. It has been a surprise to us that in spite of this, neither breakage of the lymphatic nor even small leaks of lymph have occurred unless brought about by our own manipulations in the course of the experiment.

Finally, in order to prevent coagulation of the lymph, a fine wire with a loop at the lower end about 2 mm. in diameter is dipped in dry heparin and placed in the cannula. Lymph leaving the cardiac lymphatic slowly dissolves this heparin, and clotting, always a difficulty in such work, ceases to need consideration.

While the cannulation is in progress and in order to be sure the animal is thoroughly supplied with water, 20 cc. per kilogram of Ringer's solution are given intravenously. Following such an injection, for reasons we cannot explain, lymph flow is not always rapid, but failure of flow cannot be ascribed to a lack of water in the tissues. In all cases femoral arterial blood pressure has been taken, usually with both a membrane and a mercury manometer.

Pressure measurements in the cardiac lymphatic were made by attaching a glass tube to the cannula in the cardiac lymphatic. They are thus maximal end pressures and give no idea of the normal side pressure in the vessel.

Flow of lymph was determined by pipetting lymph from the cannula into weighed tubes, usually through 10 minute periods, and then weighing. Occasionally, with free lymph flow, sampling periods were less, and with low lymph flow greater. Lymph protein and blood protein concentrations were determined by means of a Zeiss dipping refractometer, with frequent checks through micro-Kjeldahl analyses. In analysing for albumin, globulin was precipitated with 22.5 per cent sodium sulphate, filtered off, and the nitrogen determined by micro-Kjeldahl analysis, a modified Pregl apparatus being used for distillation. Globulin was determined by difference. The colloid osmotic pressures were measured by means of the Hepp micro-osmometer as described by Peters and Saslow (1939). Chlorides were determined by the method of Manery, Danielson and Hastings (1938).

EXPERIMENTAL RESULTS. *The flow of cardiac lymph.* Figure 2 illustrates an experiment showing the flow of cardiac lymph per minute and the arterial blood pressure over four hours' time. It is typical of many which show how uniformly the heart produces lymph while circulatory

conditions remain constant. The dog was a young adult, and the heart weighed 85 grams. In table 1 are found figures for the average flow of cardiac lymph taken from ten experiments which were technically entirely satisfactory. All these animals were young adults apparently in the best of health, and all had an intravenous injection of 20 cc. of Ringer's solution per kilogram of body weight prior to beginning lymph collections. The table permits no correlations between lymph flow and other possible factors such as body weight, heart weight, etc. It merely gives the average production of lymph from a single working organ profusely supplied with blood capillaries and lymphatics and under constant working conditions.

When the work of the heart is increased by intravenous injections of adrenin or ephedrine, lymph flow increases promptly and often to a sur-

TABLE 1

Average flow of lymph from the heart in ten dogs

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	WEIGHT OF HEART	BLOOD PRESSURE	AVERAGE LYMPH FLOW PER MINUTE	AVERAGE LYMPH FLOW PER MINUTE PER GRAM HEART
	kgm.	*gm.*	*mm.* Hg	*mgm.*	*mgm.*
1	8.0	77.0	142	5.20	0.0675
2	11.8	111.0	129	7.72	0.0694
3	11.8	94.0	94	8.65	0.0920
4	11.8	91.0	130	9.38	0.1030
5	14.6	114.5	112	9.76	0.0852
6	11.0	85.0	160	10.47	0.1230
7	14.0	102.0	104	15.78	0.1547
8	14.4	91.0	120	15.81	0.1737
9	11.0	97.5	140	27.27	0.2786
10	11.5	91.5	112	27.56	0.3012

prising degree. Figure 3 is a chart of an experiment in which after one and one-half hours of normal conditions an intravenous injection of 130 mgm. of sodium nitrite was given. Blood pressure and lymph flow fell slowly. Toward the end of the third hour a dose of ephedrine (13.0 mgm.) was given intravenously. There was an immediate increase in cardiac activity, blood pressure, and lymph flow. In other experiments in which ephedrine was injected, the lymph flow remained high during the period of action of the drug. As may be anticipated, exactly similar results but more transient were obtained with adrenin.

Pressure in the cardiac lymphatic. We have measured the maximal pressure of the cardiac lymph in two animals by the simple expedient of attaching a narrow glass tube to the cannula in the cardiac lymphatic.

In one instance the pressure rose to 14.1 cm. of lymph. In the second, with a mean blood pressure of 112 mm. of mercury, it was 15.5 cm. of lymph, and as a result of an intravenous adrenin injection rose to 18.6 cm. of lymph.

The composition of cardiac lymph. The amounts of lymph which can be collected in experiments such as we have described are often too small to permit a reasonably complete series of quantitations. One of our principal interests has been the percentage of protein in the lymph. This has been measured in 18 experiments on different dogs, and frequently many times in the same experiment. Our lowest figure for cardiac lymph was 2.50 per cent, the highest 4.73 per cent, with an average of 3.69 per cent.

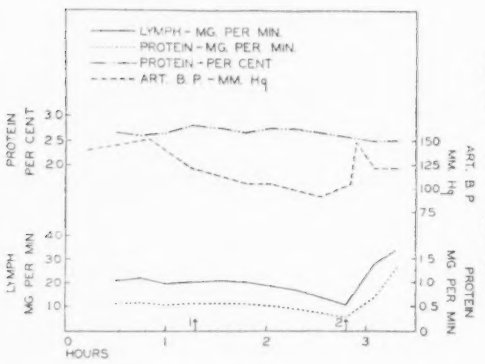

Fig. 3

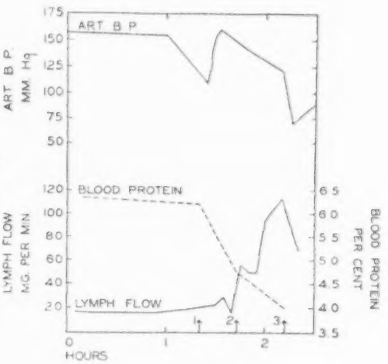

Fig. 4

Fig. 3. Cardiac lymph flow, blood pressure, and per cent of lymph protein in a dog given sodium nitrite and ephedrine. *Ordinates*, lymph flow in milligrams per minute; *abscissae*, time in hours. At arrow *1* on abscissa, 130 mgm. sodium nitrite intravenously; at arrow *2*, 13 mgm. ephedrine sulphate.

Fig. 4. Plasmapheresis and cardiac lymph flow. At the arrows *1*, *2*, and *3*, blood was removed and washed red cells reinjected.

In table 2 we have listed experiments upon which we have been able to make a number of analyses, and of the animals in this table, nos. 5, 6, 7, and 10 appear in table 1, which deals with the amount of lymph flow per minute in these animals. The discussion of certain of the implications of the figures in table 2 is best reserved until the presentation of further data. It may, however, be pointed out that cardiac lymph, like lymph from all other parts of the body, contains the blood proteins, and clots; and that the pericardial fluid, which in addition to filtration through the walls of the blood capillaries, has also passed through a considerable fraction of the epicardium or pericardium, contains the blood proteins, and

clots, and is merely a slightly diluted lymph (Maurer, Warren and Drinker, 1940).

TABLE 2

A summary of certain factors in the composition of blood, cardiac lymph, and pericardial fluid in six dogs

NUMBER OF EXPERIMENT	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN GLOBULIN	CHLORIDE	COLLOID OSMOTIC PRESSURE		Cl IN SERUM Cl IN LYMPH AND PERICARDIAL FLUID
	Blood serum							
	per cent	*per cent*	*per cent*		*mgm./100 cc.*	*mm. H_2O*	*mm. H_2O per gram protein*	
5	4.67	2.37	2.30	1.0		165	33.1	
6	6.31	3.56	2.75	1.3	418	239	37.8	
7	5.52	2.67	2.78	0.96	417	219	39.7	
10	5.36	3.50	1.86	1.9	412			
11	5.50	2.70	2.80	0.96		244	44.3	
12	8.31	3.06	5.25	0.58		302	36.3	
	Lymph							
5	2.94	1.60	1.34	1.2		134	45.4	
6	3.60	2.26	1.34	1.7	475	169	46.9	0.88
7	3.15	1.86	1.29	1.4	455	142	45.0	0.92
10	3.91	2.60	1.31	1.9	421			0.98
11	4.70	2.30	2.40	0.96		189	40.2	
12	4.70	2.57	2.13	1.2		240	41.0	
	Pericardial fluid							
5	1.12	0.58	0.54	1.1				
6								
7	1.84	1.18	0.66	1.8	438	72	39.1	0.95
10	2.66	1.12	1.54	0.7	447			0.92
11	1.20	0.75	0.45	1.7		56	46.0	
12	1.31				416	48		

TABLE 3

The appearance of horse serum after intravenous injection, determined by immunologic methods in cardiac lymph

	LYMPH + SALINE	ANTISERUM + SALINE	LYMPH				BLOOD	
			Control	Hours after injection			Hours after injection	
				1	2	3	2	3
Undiluted	–	–	–	+++	++++	+++++	++++	++++
1:50	–	–	–	+	++	+++	+++	++++
1:100	–	–	–	–	+	+	+++	+++
1:500	–	–	–	–	–	–	+	+

In order to demonstrate the permeability of the cardiac blood capillaries, we have cannulated the cardiac lymphatic and injected horse serum intravenously, and have determined the time of appearance of horse serum in the cardiac lymph.

Experiment 2. Weight of dog, 11.8 kgm.

9:00 a.m. Nembutal anesthesia.
11:40 a.m. Cardiac lymphatic cannulated.
1:40 p.m. Normal lymph and blood samples collected; 50 cc. of horse serum given intravenously without observable effect on heart or blood pressure.
4:42[30] p.m. Lymph and blood collections finished.

Table 3 summarizes the results and indicates that foreign protein readily enters the cardiac lymph from the blood just as it has been shown to do in the case of leg lymph, cervical lymph, and thoracic duct lymph after intravenous injection.

The same type of experiment has been carried out using gum acacia instead of horse serum.

Experiment 23. Weight of dog, 11.8 kgm.

9:00 a.m. Nembutal anesthesia.
10:40 a.m. Cardiac lymphatic cannulated.
1:10 p.m. Control specimens of blood and lymph collected, and 3 grams gum acacia injected intravenously. Strength of solution 7½ per cent. No effect on heart and blood pressure.
4:20 p.m. All specimens of blood and lymph collected.

Acacia in blood and lymph was determined as described by Maurer, Warren and Drinker (1940).

The results are shown in table 4.

The use of plasmapheresis and the Starling heart-lung preparation to illustrate certain features of lymph flow from the heart. The experiments so far discussed have been carried out upon animals which were in normal condition except for the anesthesia with nembutal, the open chest, and the cardiac lymphatic cannulation. The normal course of lymph flow has been altered by drug injections, but not by such radical procedures as are implicit in plasmapheresis and possible by use of the Starling heart.

Figure 4 illustrates a plasmapheresis experiment upon a dog weighing 11.5 kgm. After a long period of very steady conditions as to blood pressure and lymph flow, 650 cc. of blood were removed in two installments, as indicated by the arrows in figure 4, and the same amount of washed cells suspended in Ringer's solution was reinjected. The blood protein was reduced from 6.21 per cent to 3.99 per cent with a very great increase in lymph flow. Following a third and final blood removal and

reinjection of washed cells at 3:12 p.m., there was a serious fall in blood pressure and lymph flow—findings not infrequent in acute plasmapheresis when, after the experiment is pushed a certain distance, it seems as if the blood capillaries were damaged and for a time abnormally permeable. Before the first hemorrhage and reinjection (at 2:20 p.m.), blood protein was 6.21 per cent; preceding the second removal and reinjection (at 2:43

TABLE 4

The appearance of gum acacia in cardiac lymph after intravenous injection of 3 grams in a 7.5 per cent solution

	BLOOD	LYMPH
	mgm. per cubic centimeter	*mgm. per cubic centimeter*
Control	0	0
After 1 hour	3.0	0
After 2 hours	2.83	0
After 3 hours	2.63	0.26

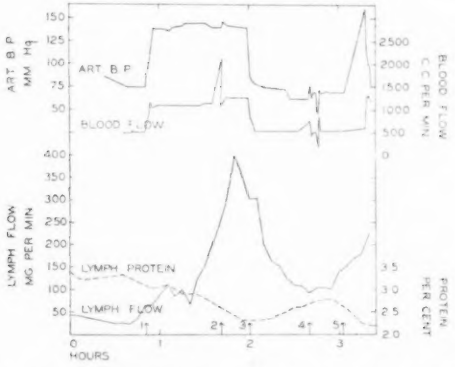

Fig. 5. Starling heart-lung experiment with cardiac lymph collection. At arrow *1*, cardiac inflow was increased and arterial resistance raised; at arrow *2*, 2.5 cc. of 1:50,000 adrenin were placed in the venous reservoir; at arrow *3*, cardiac inflow was decreased; at arrows *4* and *5*, 1000 cc. of Ringer's solution were added to the blood, reducing the blood protein from 4.68 per cent to 1.54 per cent.

p.m.), it was 4.74 per cent; and before the third removal and injection occurred (at 3:12 p.m.), it was 3.99 per cent. Serious fall in blood pressure did not take place until after the final hemorrhage, but the great increase in lymph flow shown in figure 4 takes place in the 29 preceding minutes when the blood protein was 3.99 per cent. The experiment so far as effect on lymph is concerned is entirely in accord with experiments on acute

plasmaphefesis when lymph was collected from the thoracic duct and cervical lymphatics (Field and Drinker, 1931).

We have shown the increase in cardiac lymph flow which follows the augmented cardiac activity induced by ephedrine. That experiment and others in which adrenin was used do not give any satisfactory idea of lymph flow during normal increase in cardiac work, blood flow, and arterial pressure which result from severe muscular exercise. The single way to gain an idea of maximal production of cardiac lymph is through the use of a Starling heart-lung preparation in which it is possible at will to increase the inflow of blood to the heart, to increase peripheral resistance, and to add the effects of adrenin.

In collecting lymph from a Starling heart preparation, it is advisable to section the four upper ribs and remove the sternum from the upper end to below the fourth rib articulation. This gives more space for placing the necessary ligatures around the great vessels and makes it possible to tie the aorta without disturbing the lymphatic cannula, which should be in place prior to shifting from the normal to the heart-lung circulation. We have used the superior vena cava for blood inflow and the left subclavian artery for outflow. The mechanical arrangements of the peripheral circulation were of the usual type, and heparinized dog blood was the circulating medium. Figure 5 is a chart of a Starling heart experiment with cardiac lymph collection. The dog weighed 11.3 kgm., and the heart 91 grams. The combination of increased inflow of blood to the heart, increased blood pressure, and adrenin injection caused a lymph flow of 400 mgm. per minute. It is, however, doubtful if a flow above 300 mgm. a minute could be sustained under conditions simulating what would be the circulatory situation in a dog during a long run. The possibilities for the hound engaged in a 12 hours' chase are approximately 18 cc. of lymph per hour, or 216 cc. for the 12 hours. Taking a good-sized hound, such as the subject of our Starling heart experiment, with a heart weighing 91 grams, this means 2.4 cc. of lymph per gram of heart during the 12 hours.

Discussion. The experiments which have been described seem to us to accomplish two things. First, to a limited extent, they describe a physiological mechanism in the heart which heretofore has had no attempts at measurement. What may be the significance of the lymphatic drainage of the heart in such conditions as rheumatic heart disease in children, when at autopsy the heart is so often edematous, or, if we may describe it more cautiously, seems to contain too much interstitial fluid, and what may be the significance of this system as advancing years bring on the fibrous changes in the heart called chronic myocarditis? These are problems in experimental medicine to which we can do no more than draw attention. Under normal circumstances the function of the lymphatics is obviously the same as in other parts of the body—namely, to

remove the protein-containing tissue fluid from the part, and after passage through a lymph node to return it to the circulation.

The second and to us more significant point in regard to these experiments is their bearing upon the function of the lymphatic system, and particularly the relation of lymph to tissue fluid. In brief, we have shown that in normal dogs cardiac lymph flows steadily and in small amounts. This lymph always contains protein—in 18 dogs measured under satisfactory conditions an average of 3.69 per cent, with 4.73 the highest percentage and 2.50 the lowest. These figures are comparable to protein concentration of lymph in other regions, notably, and where we have most figures, for lymph from the cervical lymphatics.

In 1931, two of us (Drinker and Field) on the basis of a number of collections of lymph taken from various parts of the body suggested "that capillaries practically universally leak protein; that this protein does not reënter the blood vessels unless delivered by the lymphatic system; that the filtrate from the blood capillaries to the tissue spaces contains water, salts and sugar in the concentrations found in blood, together with serum globulin, serum albumin, and fibrinogen in low concentration, lower probably than that of tissue fluid or lymph; that water and salts are reabsorbed by the blood vessels and the protein enters the lymphatics together with water and salts in the concentration existing in the tissue fluid at the moment of lymphatic entrance. The lymph from any given drainage area contains a varying amount of protein dependent upon the amount of water absorption which has taken place in the region from which the collection is made, and represents a cross section of the tissue fluid of the area in question." This suggestion has been criticized but never in terms of direct experiment—always through experiments in which the possible concentrations of protein in the tissue fluid have been calculated through observations upon the blood when a part has been subjected to a variety of experimental manipulations. There is no argument as to the identity of the salt content of lymph, tissue fluid, and blood. The single difficulty rests with the problem of the amount of protein in this fluid.

Since 1931, several direct experiments have appeared which bear upon the problem, and by direct we mean experiments in which tissue fluid and lymph may be compared by analysis, not by inference. First, Drinker, Field, Heim and Leigh (1934) measured the protein content of lymph and edema fluid in dogs whose legs had been made edematous by lymphatic obstruction. The results appear in table 5, and the identity of protein concentration between lymph and edema fluid needs no comment. Second, and in the same year, Weech, Goettsch and Reeves (1934), in dogs rendered edematous by plasmapheresis or by protein deprivation, showed a striking similarity in protein concentration of edema fluid and

lymph taken from the feet in a number of dogs. Table 6 illustrates their findings. Third, Maurer (1938) collected tissue fluid from frog muscle

TABLE 5

The protein content of lymph and edema fluid from dogs 1, 2, 3, and 4. Blood and lymph collected simultaneously

(Drinker, Field, Heim and Leigh, 1934)

ANIMAL	DATE	LEG	PROTEIN	
			Lymph	Edema fluid
			grams per 100 cc.	*grams per 100 cc.*
Dog 1	Feb. 25, 1933	Left	2.67	2.00
	Mar. 8, 1933	Right	2.45	2.20
Dog 2	Sept. 15, 1933	Left	3.37	3.37
	April 3, 1934	Left	2.48	3.45
Dog 3	Mar. 28, 1933	Left	2.28	2.75
	May 5, 1933	Left	2.97	3.17
	Oct. 16, 1933	Left	3.17	3.17
	April 6, 1934	Right	2.50	2.67
Dog 4	April 9, 1934	Left	2.55	1.86

TABLE 6

Comparative protein contents of edema fluid and the lymph collected immediately after cannulization

(Weech, Goettsch and Reeves, 1934)

DOG NUMBER	HIND LEGS				FORE LEGS				ASCITIC FLUID	NATURE OF EDEMA
	Right		Left		Right		Left			
	Edema fluid	Lymph	Edema fluid	Lymph	Edema fluid	Lymph	Edema fluid	Lymph		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
5	0.23	0.18								Nutritional
8-40	0.04		0.02	0.11					0.02	Nutritional
8-06	0.17	0.28	0.14	0.60		0.31		0.23	0.13	Nutritional
8-38	0.08	0.53		0.30		0.29			0.32	Nutritional
9-92						0.07		0.06	0.01	Plasmapheresis
6						0.32		0.15	0.03	Plasmapheresis
5-8	0.09	0.06	0.08							Plasmapheresis
9-1	0.86*	0.38	0.95*							Plasmapheresis
2-3			0.17	0.19	0.16	0.14				Nutritional
1-31	0.04	0.01	0.16*						0.01	Plasmapheresis

* These edema fluids contained blood. See text.

and found blood proteins invariably present. When his figures for the concentration of this tissue fluid protein are compared with the figures for protein concentration in frog lymph, there is again an identical result.

To these observations may be added those in this paper. Table 2 presents protein concentrations in the heart lymph of six dogs. The amounts of protein in the pericardial fluid of the same animals are also given, and while they are less than for the lymph, it still remains that they are far above the amounts of protein usually thought to exist in tissue fluid. Maurer, Warren and Drinker (1940) have given evidence for the belief that pericardial fluid is a simple extracellular fluid filtered from the blood capillaries. Since this fluid readily becomes bloody if the heart is manipulated, there is reason to feel that it comes from the heart, and is in fact a filtrate from the epicardial blood capillaries which passes not only through their endothelial walls, but also through the epicardial endothelium. The fact that the pericardial fluid contains the blood proteins in lower concentration than the lymph is, in our opinion, due to a double filtration—the concentration in the cardiac lymph representing the approximate concentration in the tissue fluid of the heart, and the pericardial fluid concentration representing that of the tissue fluid reduced slightly by a second filtration through the epicardium.

SUMMARY

1. A method for collecting the entire lymph flow from the heart is described.

2. Cardiac lymph flow varies directly with the vigor of the heart beat. It increases with dilution of the blood proteins and consequent enhancement of capillary filtration.

3. The composition of cardiac lymph is described in some detail in six dogs and is compared with that of the pericardial fluid.

4. The cardiac lymph is a filtrate from the blood capillaries. Normally it contains serum albumin and globulin and it clots. Furthermore, if horse serum is given intravenously, it can be detected immunologically in the lymph, and similarly gum acacia is also found in this lymph after intravenous injection.

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REACTIONS OF LARGE AND SMALL ARTERIES IN MAN TO VASOCONSTRICTOR STIMULI[1]

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It is commonly believed that the arterial circulation is controlled primarily through a flood-gate mechanism located principally in the arterioles and the small arteries supplying them. Larger arteries and main artery trunks have been considered as elastic reservoirs having little directly to do with vasomotor reactions.

The plethysmographic observations of Schretzenmayr (1) on the exposed median and large arteries of the animal established their synergistic participation in reactions in which the arterioles have been usually considered as dominant. The larger arteries were observed to react in the same direction as the arterioles in response to direct nerve stimulation, vasomotor reflexes, dilator and constrictor drugs. They were shown to be in a state of tone due to continuous vasomotor influence. Quantitative (but not qualitative) differences in the reactivity of large arteries showed regional differences but did not correspond with the classification into "muscular" and "elastic" arteries.

Encouraged by these data and the existence of selective reaction patterns in the skin (3), it seemed desirable to compare large and small artery behavior in man. The radial artery and finger pad were selected for several reasons: 1. This artery lies near enough to the surface to be conveniently recorded with the plethysmographic technique used. 2. The finger vessels are extremely active indicators of vasomotor reactions. 3. Previous study of the hand skin (3) showed uniformity in the small artery and arteriole responses over the entire hand, so that if differences occurred in the reactions of the finger pad vessels and of the radial artery, these differences would be more probably related to differences in size, and possibly innervation, than to topographical factors. 4. In addition, there seems to be general agreement (4) that the radial artery's vasomotor innervation is supplied in the same manner from the peripheral somatic nerves as is the case for the digital arteries, and that differences exist only in the abundance of the supply.

[1] This investigation has been made with the assistance of a grant to A. B. H. from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

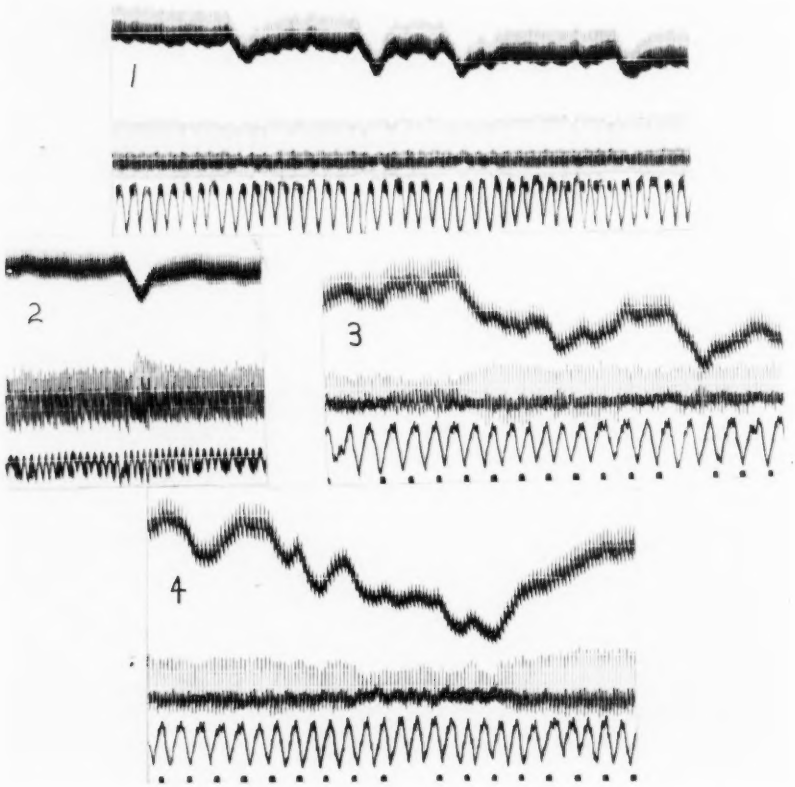
METHODS. Finger vessel reactions were recorded with the photoelectric plethysmograph previously described (2). When this plethysmograph is placed in contact with the skin over the radial artery, the volume pulses in the latter are recorded instead of skin vessel reactions, (the arterial blood supply to the skin is poor in this vicinity), since the effect of the radial pulse now dominates the amount of light returning to the photoelectric cell (2). It seemed convenient to record the radial artery volume pulses on a constant base line. This was done by employing a capacity coupled amplifier (to be described in a later paper) instead of the resistance coupled amplifier used in our plethysmographic studies. The amplitude of the volume pulse so recorded was used as a criterion of the tone of the radial artery. There is a good basis for this. There is the common clinical experience that the amplitude of the oscillations of a large artery is proportional to the patency of its lumen, that the oscillations decrease in size when the artery is constricted by spasm or by organic obstruction. It is realized, of course, that gross changes in the stroke of the heart, in arterial pressure levels, and also in the artery's distensibility with various pressure levels, will influence the amplitude of the volume pulse in the radial artery. But these influences either do not appear to contribute significantly to the experiments reported below or they are noted when they occur. Healthy male medical students served as subjects, in a semi-reclining position on a comfortable couch. Room temperatures varied between 25.0°C. and 29.0°C. in different experiments but were usually constant within one degree during the experiment.

RESULTS. Small waves, synchronous with respiration, are commonly seen in the radial volume pulse, and less frequently on the finger plethysmogram (fig. 1). They are probably due to variations in the stroke of the heart with the respiratory cycle.

Spontaneous waves are a common occurrence in the small arteries of the hand skin. They are considered as ordinarily having a vasomotor origin (3). Their absence from the radial artery (fig. 1) seems to be typical but this is not always the case. Increased amplitude in the radial volume pulse may occur at the time of the finger constriction (figs. 2 and 3). We have been unable to decide whether this is a mechanical effect from the constriction of the finger vessels, (i.e., it is due to the rise in resistance peripheral to the radial artery increasing the systolic engorgement) without change in radial tone, or whether a relaxation of radial muscle has actually occurred (fig. 3). Prolonged spontaneous waves in the fingers, probably of psychic origin, are apt to show also but to a less extent in the radial volume pulse (fig. 4). In this case, increased radial tone seems to be the explanation since changes in heart action are probably negligible here, and since the changes in finger and radial volume pulses are in the same direction. These more prolonged vasomotor discharges apparently

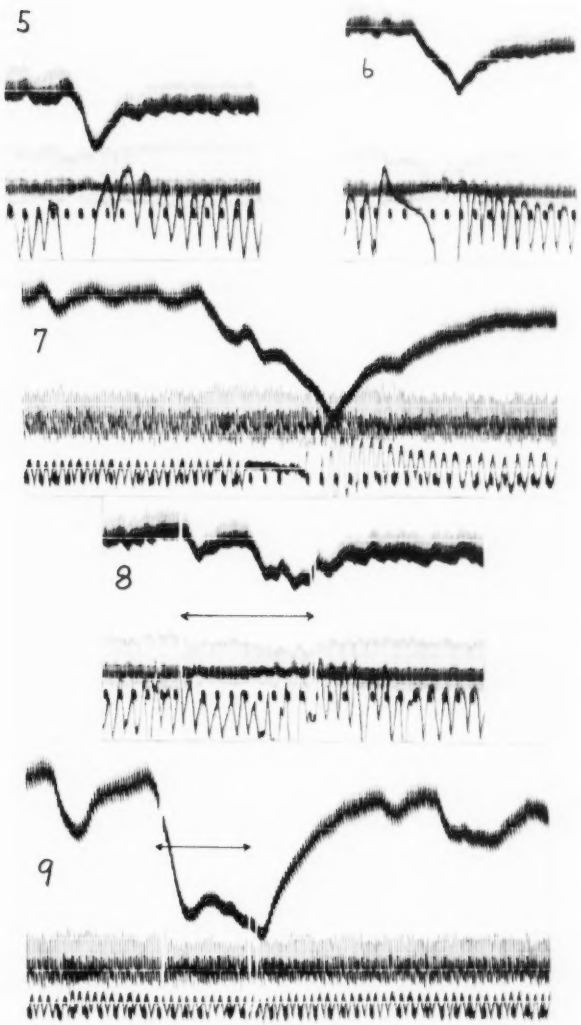
involve more vascular territory than the briefer ones, since the latter superimposed on, or preceding or following the former, often do not affect the radial volume pulse when the more prolonged discharges do.

Deep breath. The vasoconstrictor reflex in the digits from a deep breath has been shown not to involve the skin areas of the head (3). The radial



Figs. 1–4. Finger plethysmogram (upper record) and radial volume pulse (lower record). Respiration. Time = 5 seconds. Spontaneous waves.

artery may also fail to participate (fig. 5). The initial decrease in amplitude of the finger and radial volume pulses occurs simultaneously. That this is probably not due to a constrictor reflex but rather to a temporary decrease in left ventricular stroke is indicated by the increasing amplitude of the radial volume pulse at the time that precipitate fall in finger volume and volume pulse occurs. We conclude that the radial artery did



Figs. 5-9. Finger plethysmogram (upper record) and radial volume pulse (lower record). Respiration. Time = 5 seconds.

Fig. 5. Deep breath.

Figs. 6-7. Breath hold.

Figs. 8-9. Cold pressor test.

not share in the constrictor response in this instance. This seems to be the rule even in subjects who give intense responses to a deep breath in their digits.

Breath hold. This procedure has been offered as a convenient standard way of causing pressor responses (5). It elicits a more powerful and widespread vasoconstrictor discharge than does a deep breath if one may use the differences in the blood pressure responses as evidence. The breath hold causes a rise in arterial blood pressure varying in extent with the subject. Brachial blood pressure tends to fall or remain unchanged with a deep breath (6). One might expect, therefore, participation of the radial artery in the pressor response to the breath hold. Figure 6 illustrates such participation, but the constrictor effects are strikingly less than those in the finger. The initial decrease in the radial volume pulse is probably due to decreasing stroke of the heart. This shows likewise in the finger volume pulse. The beginning of vasoconstriction in the finger is timed with further progressive reduction in the radial volume pulse and the minima in both curves occur very nearly together. Recoveries likewise parallel each other in both records. Involvement of the radial artery in the constrictor response to the breath hold is, therefore, probable but it is obvious that the effects on the radial artery are relatively small. This difference in intensity of the response correlates qualitatively with differences in the richness of the vasoconstrictor innervation of the radial and digital arteries and arterioles and may well be due to such quantitative factors. That topography is an additional factor in the vasoconstrictor responses is suggested by failure of the head skin to participate (3). Likewise, failure of the radial artery to react is illustrated in figure 7 in which, despite profound constriction in the finger, the radial volume pulses are slightly increased during the finger constriction. Again, it is not possible to decide in this case whether the increased radial volume pulse is due to the rise in the resistance peripheral to the artery, or to changes in pressure levels, or actually represents decreased tone of the muscle of the artery. It would be interesting to know if the radial artery reactions are related to the extent of the rise in the arterial blood pressure in this test, but insufficient data have been obtained to attempt such a correlation.

Cold pressor test. The blood pressure responses of medical students seem to be somewhat higher in the case of the cold pressor test than in the case of the standard breath hold. This difference may be related to unrecognized small deviations from standard directions for the breath hold. The cold test presents a standard technique for presenting a painful stimulus. The vasoconstrictor responses are variable in intensity and topography as shown in the effects on blood pressure and on the circulation in various skin areas (3).

Three illustrations showing various degrees of response of the radial artery are provided in figures 8 to 10. The subjects of figures 8 and 9 gave

normal blood pressure responses. In each of these, marked constriction of the finger arteries occurred without much participation of the radial artery. In figure 8, the radial artery response parallels qualitatively that in the finger both during the constriction and also during the recovery from the procedure, but the changes in the radial artery are much smaller than those in the finger arteries. In figure 9, the profound constriction in the finger is accompanied by a very slight constriction of the radial artery. The spontaneous wave in the finger preceding the response to the cold did not involve the radial artery. In figure 10, the response of an hyper-reactor with normal resting arterial blood pressure presents a striking contrast. There was progressive constriction of the radial artery almost to obliteration. Recovery was slow, requiring five minutes to return to the control level. Yet, in this subject, the radial artery failed to share in the responses to psychic stimuli and in the spontaneous waves.

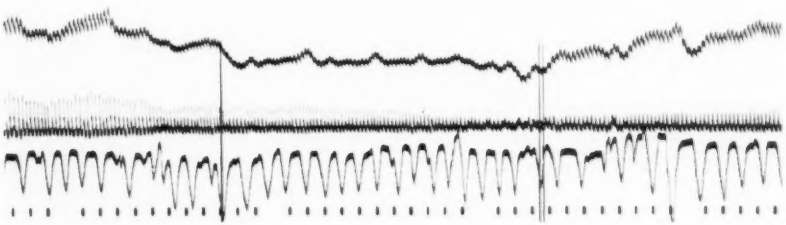


Fig. 10. Finger plethysmogram (upper record) and radial volume pulse (lower record). Respiration. Time: 5 seconds. Cold pressor test between signals.

Comment. We believe that we have shown that Schretzenmayr's thesis of synergistic participation of the large arteries in arteriolar and small artery reactions (1) is applicable only to special instances in the case of the radial-finger artery field in man. As the stimulus eliciting a vasoconstrictor reflex increases in effectiveness, and as the response involves larger vascular areas, the radial artery may finally be involved, usually only to a moderate extent.

The extent of finger constriction does not seem to be a guide as to whether the radial artery will constrict or not. Profound finger constrictions to loud noises, psychic stimuli, deep breaths are ordinarily unaccompanied by radial artery responses. Breath holds which are effective in increasing the arterial blood pressure and in producing maximal finger constrictions have only a slight constrictor influence on the radial artery. The cold test seems to be particularly effective in hyper-reactors, in effecting marked constriction of the radial artery, but it has only a moderate effect in those whose arterial blood pressure responses are normal, although the degree of finger constriction may be equally great in either type of subject.

It is possible that our method of observing the radial artery may have failed to reveal small changes in tone. Schretzenmayr's arteriograms (1) on the animal show marked quantitative but not qualitative differences in the changes in tone in the carotid and femoral arteries as compared to the large splanchnic arteries, the latter reacting much more vigorously. The reactions of the former were often overcome by the changes in blood pressure. However, his experiments differed radically from ours in that he employed powerful drugs and other procedures involving massive changes in the circulation. The two groups of experiments are not comparable nor are the two sets of data contradictory.

Our data support the possibility of selection with respect to the participation and the intensity of the participation in a given vasomotor response, of the large and small arteries in the same vascular field. They do not deny the synergistic action of large and small arteries in massive disturbances of the circulation. However, such action does not seem to be significantly involved in the more sharply localized and more moderate vasomotor reflexes studied in this paper.

SUMMARY

The possibility of synergistic participation of the large arteries in the reactions of the small arteries and arterioles supplied by them, has been studied in man, on the radial artery-digital artery field, using the photoelectric plethysmographs, previously described, to record the reactions.

The responses observed involved spontaneous waves (figs. 1–4), the reactions to a deep breath (fig. 5) to the breath hold (figs. 6 and 7), to the cold pressor test (figs. 8, 9 and 10), to loud noises and various psychic stimuli.

Radial artery participation in the vasoconstriction of the finger arteries was irregular and most obvious in instances of massive disturbances of the circulation. The degree of constriction of the finger arteries had little predictive value with respect to the occurrence of constriction in the radial artery. The data appear to show selection with respect to the participation, and with respect to the intensity of the participation, of the radial artery in the vasomotor responses of the small arteries and arterioles which it supplies.

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A STUDY OF ALLEGED QUANTITATIVE CRITERIA OF VASOMOTOR ACTION

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It is the vogue to utilize the magnitude of rise or fall in mean blood pressure induced reflexly or by humoral agents as a quantitative criterion for the degree of generalized vasoconstriction. Upon such criteria, far reaching conclusions are drawn regarding potency of hormonal agents and complicated central neural processes are inferred. This is done by eminent investigators despite the well known fact that it is not easy to evaluate the relative share that increased minute output, alterations in aortic capacity and elasticity and total peripheral resistance play in such pressor effects. Furthermore, the question whether absolute or percentile changes in blood pressure offer the better criterion of vasomotor action has never been settled (4, 5).

We have attempted to study these criteria of vasomotor power by comparing them with peripheral resistances calculated by a formula based on Poiseuille's law, viz., $R = \frac{P_m \times 1335}{V_t} = \frac{\text{dynes} \cdot \text{sec.}}{cm.^5}$, in which P_m denotes mean pressure and V_t, cardiac output per sec. For this purpose, mean carotid pressure was recorded by a properly damped Hg manometer and cardiac output was estimated by a calibrated cardiometer, carefully placed about the ventricles. Twelve dogs anesthetized with sodium barbital or amytal,[1] under mild artificial respiration, were used.

We may anticipate by stating that the investigation ended with the conclusion that the question at issue cannot be settled in this way because *peripheral resistance calculated in this way* is not solely determined by the algebraic sum of changes in size of the minute vessels. The evidence leading to this conclusion is briefly reported in this communication.

Control values. In seven dogs with standard mean pressures of 80 mm. or more, the total resistance at the start ranged from 5880 to 9080 absolute units (A.U.). This is considerably higher than estimates in man, e.g., 1150 A.U. (O. Frank), 740 A.U. (Lauber), 520–1000 A.U. (Böger), 539

[1] We are indebted to Eli Lilly and Co. for a supply of amytal for experimental purposes.

A.U. (Ranke), 1140–2740 A.U. (Böger and Wezler). (For references see Böger and Wezler (2)). The values are also higher than similar estimations on a few dogs by Böger (1) (4020–4470 A.U.) and by Broemser and Ranke (3) (2060–3100 A.U.); but much lower than estimates on rabbits by the same investigators (12,370–12,500 A.U.; 11,620–12,590 A.U.). In short, the calculated values seem to vary inversely as the size of the animal and not directly as one would expect.

In dogs, with open chests under artificial respiration, the values are not altered significantly by shock or shock-like conditions. Thus, in 3 dogs blood pressure had fallen to 46-50 mm. Hg at the start of observations, presumably due to operative shock. In these, the calculated peripheral resistance was 5249 to 7180 A.U., i.e., essentially that of dogs with normal mean pressures. This is contrary to much good evidence that peripheral resistance alters in this condition.

Reflex pressor reactions. In 22 tests, in which one or both central ends of vagus nerves were stimulated, mean pressures rose from the general average of 80 to 90 mm. Hg to ranges between 120 and 240 mm. Hg, depending on the strength of stimulation and number of nerve fibers excited. In every case, and regardless of coincident changes in cardiac output, the total calculated resistance increased with the rise in pressure. Not much more can be said. Since the initial mean pressures were so near 100 no essential differences in relation to the absolute or percentile increase would be expected, and none were found. However, when either the pressures or calculated resistances are plotted as a linear curve, the other does not follow linearly.

Since the dominant increase in resistance due to reflex pressor actions presumably occurs in the splanchnic area it seemed probable that at least equivalent increases in pressure and calculated resistance would result from occlusion of the thoracic aorta just above the diaphragm. This was never the case. Results from two illustrative experiments incorporated in table 1 show that the absolute and percentile increases in pressure following such occlusion are considerably less than is obtainable from reflex action and the percentile increase in calculated peripheral resistance is significantly smaller.

In twelve tests on animals, in which initial blood pressure ranged from 40 to 58 mm. Hg pressure, elevations of similar magnitudes were found possible. Thus, in one experiment pressures rose from 40 to 160 mm. Hg, a 300 per cent increase, and the calculated resistance rose from 2220 to 7170 A.U. Systolic output increased throughout the pressure rise. Correlation studies showed that throughout the rise, the actual elevation of mean pressure underrated the increase in resistance as calculated, but that the percentile increase overestimated it with respect to the calculated resistance.

Similar but less pronounced effects were obtained in 12 tests in which both carotids were clamped and in nine tests in which the central end of a phrenic nerve was stimulated. The latter caused elevation of blood pressure by 22 to 30 mm. Hg in all except one, in which the rise was 38 mm. Hg. This was attended by 10 to 30 per cent increase in calculated resistance and offers evidence for existence of some pressor fibers in the phrenic nerves.

Effects of epinephrine and pitressin. A dose of 2 cc., 1:50,000 epinephrine solution was injected into vagotomized dogs in nine experiments. In general, calculated peripheral resistance again increased more in those experiments in which mean pressure rose most. The quantitative relation-

TABLE 1

INITIAL PRESSURE	MAXIMUM PRESSURE	PERCENTAGE INCREASE	INITIAL RESISTANCE	MAXIMUM RESISTANCE	PERCENTAGE INCREASE	EXPERIMENTAL PROCEDURE
Experiment 1—vagotomized dog—12 kilos						
mm. Hg	*mm. Hg*		*a.u.*	*a.u.*		
90	174	93	9,900	21,800	120	Central vagus stim.
80	130	62	14,400	25,400	76	Occlusion aorta
94	140*	56	15,400	40,700*	164	Pitressin 1 unit
	160	70		24,800	61	
Experiment 2—vagotomized dog—8.5 kilos						
84	220	160	5,880	13,900	136	Central vagus stim.
86	234	172	6,570	14,960	127	
68	124	82	5,730	11,630	103	Occlusion aorta
74	154*		5,940	15,420*	161	Adrenalin 2 cc.
	182	146		13,180	121	1:50,000
46	156	240	6,800	23,100	240	Pitressin 1 unit

* Observations prior to those at maximal rise of pressure noted immediately below.

ships were not however as precise as appears at first glance. Thus, in experiment 2 (table 1) two previous tests with central vagus stimulation elevated mean pressures to 220 and 234 mm. respectively, the calculated resistances increasing from 5880 to 13,900 and from 6570 to 14,960 A.U. Following 2 cc. 1:50,000 epinephrine, the calculated resistance rose from 5940 to 15,420 A.U., but as pressure continued to rise it dropped again to 13,180 A.U. Since this was accompanied from the start by increase in heart rate and systolic discharge, i.e., by a considerable increase in minute output, one would anticipate a much greater elevation of mean pressure—both absolute and percentile—than during the central vagus stimulation. Actually mean pressure rose from 74 to only 182 mm. Hg and the percentile increase was less.

In four animals one "pressor unit" of pitressin, properly diluted, was administered intravenously. In every case the increase in calculated peripheral resistance was enormous but by no means always proportional to the elevation of pressure recorded. The data of table 1 illustrate two differing reactions: In experiment 2 a significant actual and percentile increase in pressure from previous shock levels took place and the calculated peripheral resistance likewise rose, perhaps fortuitously, by exactly the same percentile value as the mean pressure. In experiment 1, however, while both pressure and resistance increased up to mean pressures of 140 mm. Hg, the calculated peripheral resistance reached a maximum at this level and decreased significantly while mean pressure continued to rise. Comparison of changes in actual and percentile increase in mean blood pressure or actual and percentile increases in calculated peripheral resistance due to central vagus reflexes, aortic occlusion and pitressin are also shown in table 1. Anyone desiring an answer as to which of these three processes caused the greater vasoconstriction would be left in a quandary. These are only examples of numerous divergences of interpretation which would arise regarding quantitative changes in peripheral resistance induced by various drugs.

Discussion. The original aim of this research was to test further whether absolute or percentile changes in mean pressure offer the better criterion of vasomotor action (4). To this end, coincident modification of blood pressure through changes in respiration and heart rate were largely avoided by double vagotomy and by ventilating the lungs of the animal so as to just abolish natural respiratory movements. It was hoped that secondary changes in systolic discharge would prove so slight that a comparison of changes in calculated resistance with absolute and relative changes in mean pressure would prove decisive.

It was found through study of cardiometer tracings that every significant change in arterial resistance due to vasomotor action causes *some* change in systolic discharge despite such controls. Generally this increases but, especially when the pressure elevation is abrupt and large, it may decrease. Again it may decrease at first, and increase later. Direct response of the myocardium by dilatation and increased venous return, in which contraction of the spleen plays a part, both seem to be concerned. These alterations in systolic discharge combined with the observations of Wiggers and Wégria (7) that the aorta decreases actively in size and virtually becomes more elastic are not without effect on mean pressure. It was recently shown by studies on artificial models (6) that considerable variation in the elasticity coefficient of an aortic system fortunately affects both mean pressure and calculated peripheral resistance to an insignificant extent only. In this conclusion we differ from Böger and Wezler (2) who believe it plays a dominant rôle in determining mean pressure. On the

other hand, direct evidence obtainable on artificial circulation models (6) indicated that the relationship between systolic discharge and aortic capacity is a factor affecting mean pressure. Since mean pressure enters as a factor in the calculation of total resistance by the formula used it is apparent that these calculated resistances may also alter with changes in the ratio $\frac{\text{systolic discharge}}{\text{aortic capacity}}$, as well as with variations in size of minute vessels.

Such interpretations would explain many and perhaps all of our results, viz.: 1, that calculated total resistance varies inversely with the size of animals; 2, that it is not altered significantly during states of shock; 3, that it is not proportional to increase in mean pressure through agents which affect cardiac output, size of aorta, and peripheral arterioles to different degrees, and in different sequence, during the pressure rise; 4, that no correspondence exists between calculated resistances when pressures are elevated to equal levels by reflex action, hormones, etc., and 5, that the calculated resistance is less when the whole thoracic aorta is occluded than when its terminal minute vessels are constricted.

In conclusion, it becomes apparent that clarification of our notions regarding the meaning of "peripheral resistance" is needed. It is not clear at the present stage of progress whether the term should be advisedly restricted to frictional resistance occasioned through changes in size of small vessels and the viscosity of blood flowing through them or, following Böger and Wezler, whether central factors entering into formulae should also be included. If the latter, studies on artificial machines favor the view that this is not affected significantly by changes in aortic elasticity but rather by the variable relation between systolic discharge and aortic capacity.

In any event it becomes clear that the magnitude of peripheral vasomotor changes induced by nerves, hormones or chemicals cannot be assayed physiologically either by changes in mean blood pressure (actual or percentile) or by calculations of resistance in absolute units by formulae involving mean pressure as a factor.

SUMMARY

1. By use of simultaneous cardiometric records of the ventricles and mean arterial pressure in mildly ventilated, vagotomized dogs, data were obtained for calculating peripheral resistance changes according to the formula $R = \frac{\text{mean pressure}}{\text{cardiac output per sec.}}$ in $\frac{\text{dynes} \cdot \text{sec.}}{\text{cm.}^5}$, and comparing such values with the absolute and percentile changes in mean pressure.

2. Pressor effects on mean arterial pressure were induced in normal and "shock" dogs by reflex stimulation of the central vagus and phrenic

nerves, by bilateral carotid compression, by use of epinephrine and pitressin.

3. The results, supported by previous tests on artificial circulation machines strongly suggest that the quantitative estimation of changes in vasomotor tone of smaller vessels either by absolute or percentile changes in mean arterial pressures or by mathematical calculation of peripheral resistance is highly restricted even in experiments in which cardiac output is also measured. Mathematical calculations fail because mean arterial pressure, which enters into the formula, is affected by the relationship between systolic discharge and aortic capacity—but not, in our experience, by aortic elasticity. The formula does not take into account the aortic capacity in relation to systolic discharge either in the same animal or in animals of different sizes

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THE RELATION OF TRANSPORTATION FORCE TO MOTILITY IN THE COLON OF THE DOG[1]

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It is common practice in the study of colon motility by the balloon technique to guy the system in place in order to prevent the shifting of the balloon to a lower level during the course of an experiment. Frequently, in the course of removing a balloon system at the close of an experiment, a very appreciable force is noted to oppose the withdrawal. This force, if allowed to act unopposed, would carry the balloon system to a lower level in the colon (1). In previous work we have observed the shifting of a balloon to the extent of several centimeters in the course of a 200 minute experiment (2). Such transportation is obviously the result of a force exerted by the colon. Previous work has revealed that transportation in the colon is intimately related to a certain portion of an active period. It seems reasonable then that the force exerted would be greatest during this portion of the period. On the other hand the force exerted against the balloon may be more evenly distributed than transportation within a given period, but without significant effect because of obstruction in the lower segment. To study the latter possibility, the force exerted in an attempt to move material in the lumen of the colon must be measured by as nearly an isometric technique as possible.

To record the force related to transportation efficiency, a technique was devised in which the carbon pile connected to a galvanometer was used to indicate the extent of force. This force, tending to transport contents of the colon to a lower segment was then correlated with motility. The single balloon technique previously described (2) was used to obtain records of motility. The carbon pile used for obtaining evidence of force involved in transportation was connected by a linen thread to the junction of the balloon and tube of the motility recording system (fig. 1). A glass syringe was attached to the galvanometer in such a way that the plunger could be moved simultaneously and to an equal extent with the indicated galvanometer deflection. With the syringe connected by way

[1] The authors are indebted to Dr. A. J. Carlson, who made this study possible.

of a rubber tube to a water manometer it was possible to record even slight evidence of transportation force immediately beneath the motility tracing which indicated pressure changes in the balloon.

Data for correlating colonic motility with transportation force were obtained from five cecostomized dogs, which prior to this study had been trained to lie quietly for several hours at a time on a cushioned table. The balloon with its connections to a water manometer and carbon pile was inserted into the colon by way of the cecostomy to a depth of 10 cm.

Fig. 1

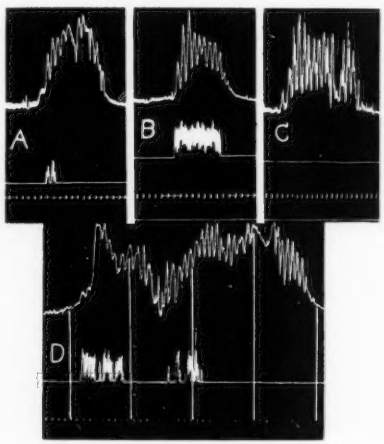

Fig. 2

Fig. 1. Apparatus used for recording colon motility and transportation force. 1 = carbon pile, 2 = arm of carbon pile, 3 = battery, 4 = galvanometer, 5 = syringe, 6 = syringe connection to water manometer, 7 = linen thread connection between arm of carbon pile and balloon, 8 = balloon, 9 = connection between balloon and manometer.

Fig. 2. Top line = balloon tracing of colon motility. Middle line = syringe record indicating galvanometer deflection as a result of transportation force. Bottom line = time in minutes.

beneath the skin. The linen thread connection between the carbon pile and the balloon was then made taut, and the balloon inflated according to a standard method (2) previously described.

Any force exerted upon the balloon tending to displace it to a lower level of the colon made more taut the linen thread connected to the arm of the carbon pile and gave as a result a deflection of the galvanometer. Each deflection was measured by means of the syringe previously described and recorded simultaneously with its occurrence. A tracing thus obtained recorded at each instance motility and its associated transportation force or "pull pattern," throughout the course of each experiment.

This method of obtaining a correlation between the transportation force and motility is more significant from a qualitative than a quantitative viewpoint. Qualitatively the exact relation could be followed between any force acting to displace the balloon and the type of motility recorded at that instant. Quantitatively, with a view to exact measurements on the extent to which the transporting force was acting was not practical with this apparatus, although estimates occasionally indicated as much as 200 grams of pull. Since, by this technique, the qualitative relation is more significant than the quantitative measurements, the term "pull pattern" may be more suitable than transportation force.

In a total of 25 experiments, each of 200 minutes' duration, a transportation force was observed only twice unassociated with activity. This may be explained as in a previous paper (2) by a consideration of force which may have been exerted upon the tube above the balloon either directly or indirectly through colon contents. A pull pattern was observed in 59 per cent of the 161 active periods recorded. These active periods

TABLE 1

The relationship between the average number of minutes of activity and transportation force per 50 minute period

	50 MINUTE PERIODS			
	1	2	3	4
Activity per 50 minutes	28	30	29	28
Transportation force per 50 minutes	3.9	4.5	5.2	3.2

varied in duration from 1 to 104 minutes. Of the active periods 54 per cent were less than 12 minutes in duration, while 83 per cent were less than 24 minutes. These figures are in striking agreement with those previously reported (2).

A tabulation of the data (table 1) relative to the motility in each 50 minute period showed activity to be present 56 per cent of the time in the first period, 60 per cent in the second, 58 per cent in the third, and 56 per cent in the fourth period. The pull pattern was evident 8 per cent of the time in the first 50 minute period, 9 per cent in the second, 10 per cent in the third, and 6 per cent in the fourth period. From the data it is obvious that a pull pattern existed in a relatively small percentage of the time as compared to that occupied by activity.

Gross observation revealed a close relationship between the active period and the appearance of transportation force, while a tabulation of data shows a wide discrepancy between the duration of an active period and duration of transportation force. A more detailed analysis, therefore, was necessary to determine the more intimate relation of the pull pattern

to the active period. This analysis was obtained by dividing each active period into four equal parts and tabulating the duration of transportation force in each quarter. Thirty-three per cent of the total transportation force appeared in the first quarter of the active period. This percentage was increased to 36 per cent during the second quarter and reduced to 19 per cent and 11 per cent respectively during the third and fourth quarters. This particular observation is a partial confirmation of previous work (2), namely, that transportation during the first half of an active period (fig. 2, part A) is greater than during the second half. The fact that the transportation force is as great in the second quarter as in the first, in contrast to our previous findings relative to transportation, is probably due to the technique employed which did not permit the balloon to descend.

Although the pull pattern is practically always related to an active period, the intensity of the active period is not an invariable index to

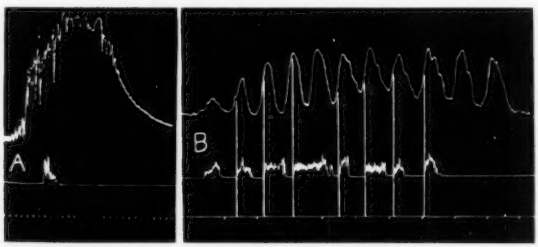

Fig. 3. Part A = Transportation force is correlated with the systolic phase of the type III contraction.

Part B = Rapid drum-showing that the start of the "pull pattern" occurs during the systolic phase of the type II contraction.

the transportation force. Periods of activity of low intensity (fig. 2, part B) as indicated by pressure changes in the balloon are often associated with a pull pattern of considerable length. On the other hand, active periods of as great an intensity (fig. 2, part C) may have no associated pull pattern. During the course of an active period one or more type III contractions may appear. Since the pull pattern seems to be related to the type III period (fig. 2, part D) such an active period which contained more than one type III contraction would tend to have the pull pattern spread more evenly throughout the period. These facts are comparable to previous observations on transportation in which the balloon was free to move.

Since the pull pattern (fig. 3, part A) was most evident during the systolic phase of a type III contraction, the speed of the kymograph was increased in order to relate the transportation force to the different phases

of a single type II contraction. In the great majority of instances (fig. 3, part B) the beginning of a pull pattern was found to be definitely related to the systolic phase of a type II contraction.

The data obtained from 17 charts, each of 75 minutes' duration, revealed that 67 per cent of the pull patterns began during the systolic phase of type II contractions. Seventeen per cent started at the peak of type II contractions, while only 10 per cent began in the diastolic phase. Occasionally the tone of the colon was sufficiently high to practically obliterate the appearance of rhythmic contractions. Only 6 per cent of the total pull patterns started during this time. The low quantity of transportation force present during high colon tone is in keeping with the evidence presented by Quigley, Highstone and Ivy (3) that high intestinal tone is not conducive to the transportation of a bolus, and confirms our previous observation (2) on the transportation of a balloon in the colon.

SUMMARY

1. A technique is described by which a force acting to displace an object in the colon could be correlated with the motility at that instance.

2. Transportation force in the colon is intimately related to the active period.

3. The time during which transportation force is exerted upon an immovable object in the colon is greatest during the first half of an active period, and least during the last quarter.

4. The intensity of an active period is not necessarily indicative of the quantity of transportation force.

5. The systolic phase of type II and type III contractions is more efficient in exerting a transportation force than other phases.

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DECUSSATION OF THE SACRAL AUTONOMIC PATHWAYS OF THE BLADDER FROM THE HYPOTHALAMUS[1]

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During recent years, the brain stem and the spinal cord have been extensively explored in an attempt to locate the reactive regions for the contraction of the bladder (Kabat, Magoun and Ranson, 1936; Wang and Ranson, 1939a). Its efferent pathway has further been delimited by stimulating the anterior hypothalamus with lesions in the different regions of the brain stem and spinal cord (Wang and Ranson, 1939b). It was found that the response is generally not abolished if the lesion is limited to one side. Evidently, the sacral autonomic pathway of the bladder is partially crossed, but the location and extent of crossing are not clear (Gruber, 1933). The present investigation was undertaken to determine more accurately the level or levels of crossing.

METHODS. Chronic unilaterally and bilaterally hemisectioned cats were prepared. The operation was achieved through the dorsal approach under intravenous nembutal anesthesia (40 mgm. kgm.). Five levels were chosen: midbrain, medulla, upper cervical cord, lower cervical cord and lumbar cord. In the midbrain hemisections, the lesions were made in a plane extending from the inferior colliculus dorsally to the rostral part of the pons ventrally. The second hemisection at another level, if desired, was done about 2 to 3 weeks after the first operation. The animals were observed frequently for bladder and other visceral disturbances.

Experiments were performed on some 30 cats three or more weeks after the final operation. At this time all the animals were in good physical condition. Light nembutal anesthesia was used (20 mgm. kgm., intravenously). A suprapubic incision was made to expose the urethra, into which a glass cannula connected with a tambour was inserted. Both hypogastric nerves were cut, the wound was then closed and 25 cc. of warm saline were injected into the bladder. Blood pressure was recorded from one of the carotid arteries by means of a mercury manometer.

The cat was then placed in the prone position, the skull trephined and bipolar bare-tipped nichrome wire electrode introduced into the hypo-

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thalamus with the aid of the Horsley-Clarke instrument. The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 V) in the primary and the secondary coil at 9 cm. The hypothalamus immediately caudal to the optic chiasma and at the level of the median eminence was explored millimeter by millimeter.

After the experiment was completed, the animal was perfused with 10 per cent formalin and the level of the lesion in the spinal cord determined. The hypothalamus and the spinal cord and or the brain stem at the level of the hemisection were fixed in 10 per cent formalin, embedded in nitrocellulose and stained by the Weil method. The point of stimulation that yielded maximal responses and the extent of the hemisection were determined microscopically from a study of serial sections.

Results. *Unilateral hemisection.* Animals with unilateral hemisections at the level of the midbrain, medulla or spinal cord showed little in the way of viscero-motor disturbances. For the first few days, there was usually a slight miosis and relaxation of the nictitating membrane on the side of the lesion, but all showed much improvement and after a month or so the differences in the size of the pupils and in the extent of relaxation of the nictitating membranes were almost imperceptible. All of these animals could micturate normally. Stimulation of either side of the hypothalamus yielded both pressor and bladder contractions in every case except cat 9 (see table 1). In cats 5 and 8 the hypothalamus on the normal side was stimulated again after section of all the sacral roots on that side. Good bladder responses were obtained (fig. 1).

Bilateral hemisections. With bilateral hemisections through the midbrain on one side and the upper cervical cord on the other, the animals behaved like the former group and could all urinate voluntarily. However, if the hemisections were placed at the levels of the upper and lower cervical cord, or of the cervical and lumbar cord, the animals were unable to micturate normally at will. "Autonomic bladder" with almost constant overflow would develop such as seen in animals with complete cord transections. For this reason, the urine was pressed out twice a day in order to prevent the formation of ulcers in the perineum. In addition, stimulation of the hypothalamus in this group of animals (cats 16 22, table 2) failed to yield bladder contractions although spontaneous rhythmic contractions in no way connected with the stimulation were often seen (fig. 2). On the other hand, stimulation of the hypothalamus in the animals with hemisection through the mesencephalon on one side and through the upper cervical cord on the other (cats 12 15, table 2) resulted in bladder contractions, and in two of them both halves of the hypothalamus were equally active.

Pressor responses were obtained in all animals with one of the hemisections placed in the midbrain or the lumbar cord. The hypothalamus

TABLE I

Bladder and pressor responses following stimulation of the anterior hypothalamus in the chronic unilaterally hemisectioned cats

CAT NUMBER	LEVEL OF HEMISECTION	MAXIMUM CONTRACTION OF THE BLADDER		RISE OF BLOOD PRESSURE IN THE CORRESPONDING BLADDER REACTIVE REGION	
		Normal side	Hemisectioned side	Normal side	Hemisectioned side
		*mm.**	*mm.**	*mm.* Hg	*mm.* Hg
1	Midbrain	29	25	7	10
2	Midbrain	19	17	14	12
3	Midbrain	15	14	14	20
4	Medulla	21	18	23	20
5	C_2	25	23	56	56
6	C_2	25	25		
7	C_3	31	23	34	32
8	C_5	27	16	27	30
9	C_5	39	0	44	18
10	L_5	27	25	44	44
11	L_7	30	26	34	34

* The measurement refers to the height of the contraction of the bladder as indicated by the lever attached to the tambour and has no absolute quantitative meaning. In the individual experiment, however, the larger the value, the greater the force of the contraction of the bladder.

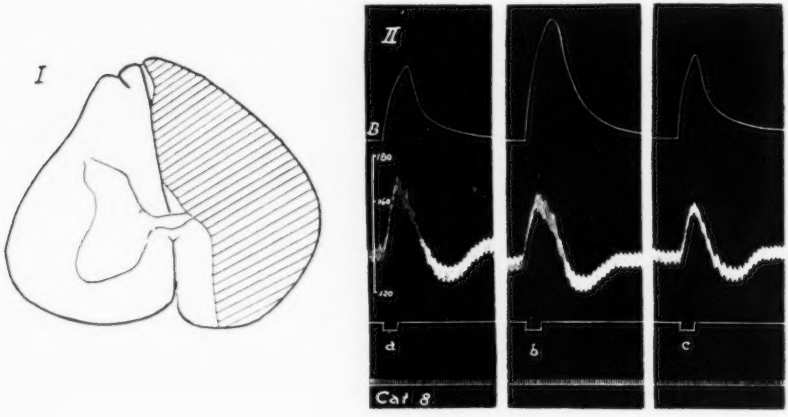

Fig. 1. I Transverse section through the spinal cord of cat 8 at the level of C_2, the extent of lesion being shown by oblique lines.

II Kymograph records of responses to hypothalamic stimulation in the same cat. Record *a* was taken from that of the hemisectioned side; *b*, from that of normal side, and *c*, from the same point as that from which *b* was taken after section of all the sacral roots of the ipsilateral side. Tracings from above downward represent bladder (B), blood pressure, the signal and time in seconds.

TABLE 2

Bladder and pressor responses following stimulation of the anterior hypothalamus in the chronic bilaterally hemisectioned cats

CAT NUMBER	LEVELS OF HEMISECTIONS	MAXIMUM CONTRACTION OF THE BLADDER		RISE OF BLOOD PRESSURE IN THE CORRESPONDING BLADDER REACTIVE REGION	
		Upper hemisectioned side	Lower hemisectioned side	Upper hemisectioned side	Lower hemisectioned side
		mm.	*mm.*	*mm.* Hg	*mm.* Hg
12	Midbrain-C_2	29	26	14	18
13	Midbrain-C_1	26	25	8	23
14	Midbrain-C_2	Trace?	35	20	24
15	Midbrain-C_1	0	33	?	10
16	C_2-C_6	0	0		
17	C_1-C_7	0	0	26*	28*
18	C_2-C_7	0	0	0	0
19	C_4-L_3	0	0	24	62
20	C_5-L_2	0	0	28	38
21	C_5-L_5	0	0	30	40
22	C_7-L_3	0	0	42	68

* The rise of blood pressure in this case was similar to that obtained on the spinal cats (Clark and Wang, 1939). They were delayed and sustained responses.

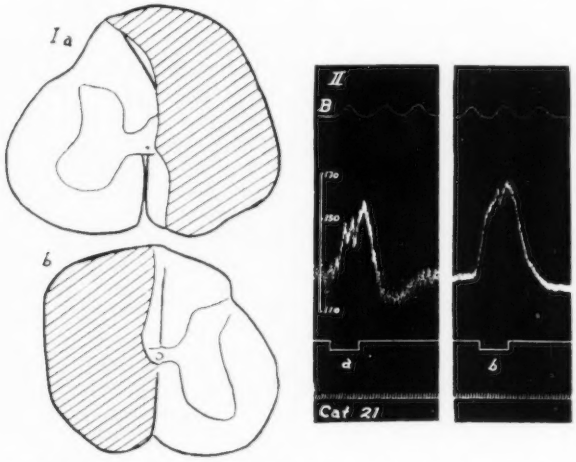

Fig. 2. I Transverse sections through the spinal cord of cat 21 at the level of C_5 (above) and L_5 (below), the extent of lesions being shown by oblique lines.

II Kymograph records of responses to hypothalamic stimulation in the same cat. Record *a* was taken from that of the cervical (C_5) hemisectioned side, and *b* from that of the lumbar (L_5) hemisectioned side. Tracings from above downward represent bladder (B), blood pressure, the signal and time in seconds. Note the spontaneous rhythmic contractions of the bladder, which were often seen in chronic transectioned cats.

in the group with the midbrain lesion (cats 12–15) was generally less reactive, particularly in cat 15. In cats 17 and 18, the hemisections were placed at the level of the upper and lower cervical cord. No prompt pressor effect was obtained. Blood pressure was not taken in cat 6 (table 1) and cat 16 (table 2).

Discussion. It is difficult to section exactly half of the spinal cord; it is even more difficult to ascertain how much of the cord is functionally intact, especially if the section is made acutely. For these and perhaps other reasons, Spiegel and MacPherson (1925) could not decide whether or not the bladder impulse crosses in the cervical and thoracic cord. However, we are now certain that the bladder impulse descends only in the lateral white columns and not in the dorsal and ventral white columns (Wang and Ranson, 1939b). Furthermore, in this experiment, narrow strips bordering the median sulci of the spinal cord are definitely shown to have no correlation with the descending bladder impulse (fig. 2). The procedure of preparing chronic hemisection, on the other hand, would certainly eliminate the acute effect of trauma (edema, etc.) on the intact side. It appears to us also that severance of both hypogastric nerves is indeed necessary, especially in case one of the hemisections is placed in the lower lumbar cord. It is known that the hypogastric nerves formed largely from the white rami of the second to fifth lumbar segments contain some motor component to the bladder (Gruber, 1933), stimulation of which may yield a small contraction of the bladder. Such responses may be easily interpreted as the crossed component of the sacral autonomic system.

Difficulty was encountered in maintaining cats with hemisection at the level of the medulla. In the single experiment with section in the medulla, a large portion of the medial reticular formation on the sectioned side was found to have been left intact. Nevertheless, the case is included in table 1 because this structure does not carry any fibers mediating impulses to the bladder (Wang and Ranson, 1939a). At the level of the midbrain, the situation is quite different. Not infrequently, the contraction of the bladder was produced by stimulating the region bordering the midline (Kabat, Magoun and Ranson, 1936; Wang and Ranson, 1939a). For this reason, a number of experiments had to be discarded for the lesion was not extensive enough to exclude all the reactive region on the side of the section. In the few experiments here reported, some midline structures have also been left uncut, but it is doubtful if they have any significance for mediating the bladder impulse.

All the animals with unilateral hemisections with the exception of cat 9 showed decussations between the hypothalamus and the level of hemisection. The most rostral decussation is demonstrated in cats 1–3, that is, a cross connection exists anterior to the midbrain lesion. In two cats

with cervical hemisections (cats 5 and 8), the bladder remained reactive to stimulation of the hypothalamus on the normal side after the sacral roots on its side were cut (fig. 1). There must therefore be another crossed connection caudal to the cervical lesion. The experiments with double hemisections at the levels of the upper and lower cervical cord or the cervical and lumbar cord, gave no evidence of any decussation at levels between C_2 and L_5. The lower decussation, then, is located caudal to the lumbar section. In the case of cats 12 15 with hemisection of the midbrain on one side and of the cervical cord on the other, a decussation at some level between the two lesions is demonstrated. We have no satisfactory explanation to offer in the case of cat 9 and of cat 15, in which no decussation is demonstrated rostral to the cervical cord and midbrain respectively. The points of stimulation are identified at the desired level and they are symmetrical; the lesion is also rightly placed. In a single experiment to show the effect of unilateral section of the cord on the contraction of the bladder induced by hypothalamic stimulation (Magoun, Ranson and Hetherington, 1938), the reaction was abolished to stimulation of the hypothalamus on the side of the lesion and was retained on the opposite side. There might be some variation in animals of the same species.

These multiple crossed connections at first seem superfluous. However, work reported elsewhere indicates that they exist. Kabat, Magoun and Ranson (1936) found that stimulation of the midline structures at the level of the diencephalon gives rise to good contractions of the bladder and suspected crossing in Meynert's and the supramammillary commissures. Barrington noted in 1925 that cats with bilateral lesions in the tegmental region of the pons ventral to the superior cerebellar peduncles showed permanent inability to empty the bladder, and therefore postulated the existence of a micturition center in that region. In the present series of experiments, the nature of the decussation at these levels is not clear; it may be a multiple one in the diencephalon and the pons, or a diffuse one in that region. As to the crossing in the lumbar region, numerous investigators have agreed that a reflex center chiefly concerned with micturition is located between the second and seventh lumbar segments (Gruber, 1933). And it is known that the centers there are connected with commissural fibers, and under ordinary circumstances the two halves act in harmony (Griffiths, 1895; Stewart, 1899). On the other hand, the absence of crossing in the entire spinal cord down to the upper lumbar segment is indeed very striking. Stewart (1899) who stimulated the upper cervical cord with a needle electrode found no response of the bladder if the cord is hemisectioned below the stimulated region on one side and in the thoracic cord on the other. Possibly because the cord was acutely hemisectioned, his negative findings have not apparently been

taken seriously. We have the advantage of not only using a chronic preparation but also stimulating a region which is more remote from the lesion. The fact that those cats cannot micturate voluntarily further substantiates our conclusion that the bladder impulses do not cross in the entire spinal cord down to the upper lumbar segment.

The pressor impulse descends from the hypothalamus and crosses partially in the brain stem and the spinal cord below the cervical region. This is in line with the evidence presented by Harrison, Wang and Berry (1939) on the unilaterally sympathectomized cats. In addition, our data indicate that there is no decussation of the pressor impulse in the cervical cord. And the delayed and sustained rise of the blood pressure in cat 17 is probably of a hormonal origin (Clark and Wang, 1939).

CONCLUSION

The anterior hypothalamus has been stimulated in chronic unilaterally and bilaterally hemisectioned cats for the contraction of the bladder. It is found that there is no decussation in the entire cervical, thoracic and upper lumbar cord. Cross connections are, however, located in the brain stem and the lower lumbar segments. The decussation in the former, that is, the brain stem, may be either multiple or diffuse in nature.

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FURTHER STUDY ON THE GASTRO-INTESTINAL MOTILITY FOLLOWING STIMULATION OF THE HYPOTHALAMUS[1]

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The numerous attempts to determine the effect of the hypothalamus on gastro-intestinal motility have led to conflicting and contradictory observations (Sheehan, 1940). By stimulating the hypothalamus in the unanesthetized cats, Kabat, Anson, Magoun and Ranson (1935) observed under fluoroscope immediate cessation of peristalsis and loss of tone of the stomach and small intestine. Beattie (1932a), Beattie and Sheehan (1934) and Heslop (1938b), using various methods such as naked-eye observation, x-ray and other recording means, found an excitatory effect on stimulation of the anterior hypothalamus and inhibition only limited to that of the posterior region. Masserman and Haertig (1938) on the other hand, reported that inhibition was obtained with a stronger current, while weak stimulus always yielded an excitatory response. The present study is conducted to supplement the previous observation made in this laboratory by exploring the entire hypothalamus to uncover its various effects on the stomach, intestine and colon, under a different set of conditions.

METHODS. With the aid of the Horsley-Clarke stereotaxic instrument, responses of the gastro-intestinal tract to faradic stimulation of the hypothalamus from the supraoptic region to the mammillary bodies were observed. In the first series of some 30 cats, light ether anesthesia was administered through a tracheal cannula. A midline abdominal incision was made and the abdominal muscles were tied around the neck of a shallow glass container. Suspension of the animal in the upright position was made by support of the head through the frame of the stereotaxic instrument and by strings passing under the supra-spinous ligament. Part of the small intestine and colon fell naturally into the container, in which warm Locke's solution was kept at 39 to 40°C. It was later found to be more convenient to observe the intestinal movements through an elliptical window made of transparent x-ray film with animal lying on

[1] Aided by a grant from the Rockefeller Foundation.

its back. The window and the abdominal wall were raised so that part of the gastro-intestinal tract could be seen.

At first animals were given about 100 cc. milk 3 hours before the acute experiment. In such a preparation, the gastro-intestinal tract usually showed occasional outbursts of activity, chiefly in the form of peristalsis. Difficulty was encountered in differentiating the spontaneous activity from that which resulted from stimulation. Inasmuch as a definite excitatory effect was often obtained during our early exploration, we confined our present study to its motor effect by choosing to use fasting animals.

In another series of 20 cats, chloralose was used instead of ether. A dose of 70 mgm. per kilo body weight, injected intravenously, was usually enough to keep the animal quiet. Balloons of various sizes were inserted into the stomach, small intestine and colon, and connected to water manometers for recording the tone and motility of each respective organ on a slowly moving kymograph. In all these experiments, the stimulus was given through a bipolar nichrome wire electrode connected to the secondary coil of a Harvard inductorium with a dry cell (1.5 V) at the primary. The distance between the primary and secondary coils varied from 9 to 12 cm. In 4 cats the spinal cord was sectioned at the upper thoracic segment after a positive response was demonstrated. Bilateral vagotomy was done in 9 others.

The anterior hypothalamus of 7 chronic cervical spinal cats was similarly explored. Kymograph records of the intestine and colon were taken in 4 of them and visual observations made in the rest.

The sites of stimulation in the hypothalamus were checked histologically with sections stained by Weil's method. The reactive points were plotted in 4 representative levels (figs. 1–4).

Results. The intestine and colon of the fasting animals were generally quiet. There was no essential difference in the results obtained with light ether and chloralose anesthesia. When the anterior hypothalamus was stimulated with a weak but effective stimulus, usually for a period of 15 to 60 seconds, there appeared at first some definite blanching of the small intestine. The color returned immediately after the cessation of the stimulus; sometimes, it appeared more reddish than before. And a short while later, there not infrequently occurred swinging movements, sometimes segmentation and occasionally peristalsis. In most instances, the responses occurred in a few intestinal loops, and only in exceptional cases were the movements generalized. The colon appeared to be more responsive and peristaltic waves were more frequently observed (fig. 5). A second stimulation of the same region usually brought about an equal or a smaller response, depending upon the length of the interval between stimuli. Generally speaking, 10 to 15 minutes was found to be an adequate interval. When a response was obtained the second time, it usually

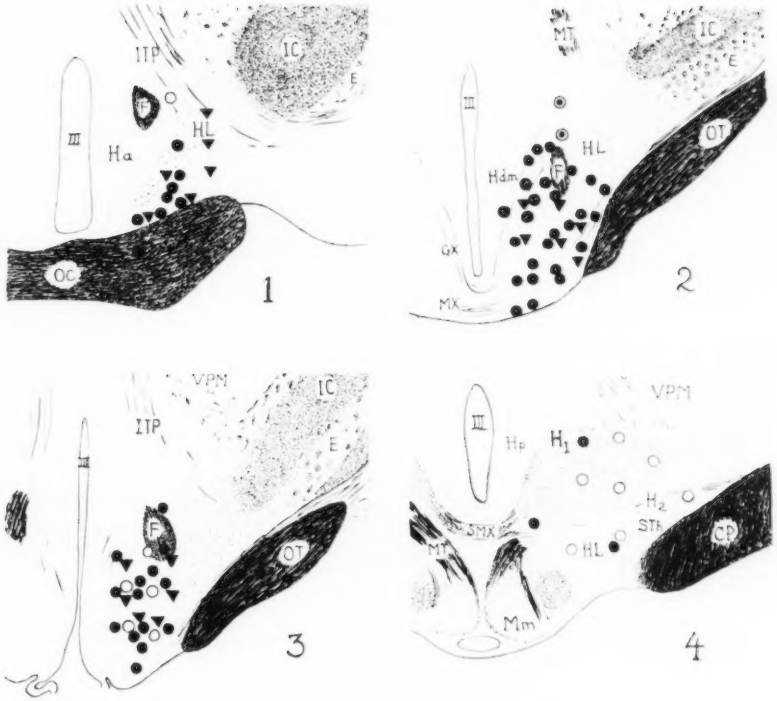

Fig. 1. Transverse section of the hypothalamus at the level of the optic chiasma. This figure includes the data from 7 cats in which various responses were obtained in this plane.

Fig. 2. Transverse section of the hypothalamus at the level of the supraoptic commissures. This figure includes the data from 13 cats in which various responses were obtained in this plane.

Fig. 3. Transverse section of the hypothalamus at the level of the median eminence. This figure includes the data from 11 cats in which various responses were obtained in this plane.

Fig. 4. Transverse section of the hypothalamus at the level of the mammillary bodies. This figure includes the data from 6 cats in which various responses were obtained in this plane.

Symbols and abbreviations for the figures 1–4

The four figures represent sections of the hypothalamus taken from four different cats. Solid circles (●) represent delayed excitations of the gastro-intestinal tract following unchanged activity during stimulation. Solid triangles (▼) represent delayed excitations following inhibitions during stimulation. Solid circles with circumscribed circles(◉) represent delayed excitation following immediate excitation during stimulation. Empty circles (○) represent excitation during stimulus. *CP*, cerebral peduncle; *E*, nucleus entopeduncularis; *F*, fornix; *IC*, internal capsule; *ITP*, inferior thalamic peduncle; *GX*, Ganser's commissure; H_1, H_1 field of Forel; H_2, H_2 field of Forel; *Ha*, anterior hypothalamic area; *Hdm*, nucleus hypothalamicus dorsomedialis; *HL*, nucleus hypothalamicus lateralis; *Hp*, nucleus hypothalamicus posterior; *Mm*, nucleus mammillaris medialis; *MT*, mammillothalamic tract; *MX*, Meynert's commissure; *OC*, optic chiasma; *OT*, optic tract; *SMX*, supramammillary decussation; *STh*, subthalamicus nucleus of Luys; *VPM*, nucleus ventralis posteromedialis; *III*, third ventricle.

occurred in the same few loops of intestine with the same latent period and the response lasted for approximately the same length of time. If the electrode was moved 1 mm. upward or downward, the intensity and duration of the response might vary, but the latency appeared to be fairly constant. In general, the latent period was 40 to 60 seconds (average, 52). It depended somewhat upon the length of stimulus; the longer the stimulus, the longer the latent period. In most cases, the excitatory responses would not appear before the stimulus was terminated. On the other hand, there were cases in which the excitatory response appeared in the midst of a prolonged stimulus and the latent period was not much lengthened. The response, the increase of tone and movement as repre-

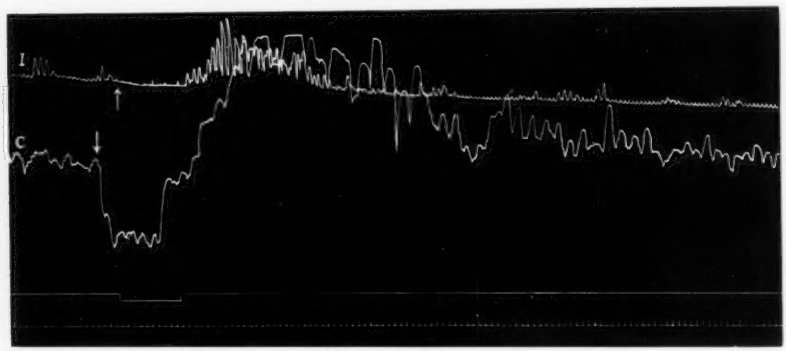

Fig. 5. Kymograph tracings showing the delayed excitatory responses of the small intestine (I) and colon (C) with loss of colonic tone during stimulus. Arrows indicate the commencement of stimulus. Time in 6 seconds. Small excursions of the tracing (I) are respiratory.

sented in figure 5, was always gradual in onset. It might take a minute or two to reach maximum and vanished so slowly and smoothly that sometimes it was difficult to make out the total duration of response. On the whole, it could be said that it lasted for 2 to 7 minutes, or longer in a few exceptional cases. Bilateral vagotomy in 6 of these cats was found not to abolish this type of delayed excitatory response (fig. 6).

As the intestine and colon were quiet under the control condition, it was often difficult to observe any inhibition. In a few cases in which there was spontaneous activity, stimulation did not invariably obliterate the regular peristaltic waves. In such cases, stronger stimulus did not alter the response, except perhaps the initial blanching was more marked. On the other hand, there were cases with inhibition of tone and other activities during stimulus, but almost always superseded later by increased tone

and contractions (fig. 5). Uncomplicated inhibition was only occasionally observed.

On account of its anatomical location, movement of the stomach was not constantly observed through the window. In 15 cats, a balloon was inserted into the stomach through the esophagus. In many cases, the change during and following stimulus was small and complicated by the vigorous respiratory movements. In 2 cats in which the response of the small intestine and colon was very marked, the stomach showed also a similar delayed excitatory response. The effect in these 2 casts was sustained for more than 15 minutes.

When the hypothalamus at the level of the infundibulum and mammillary bodies was stimulated, sometimes an entirely different type of response was

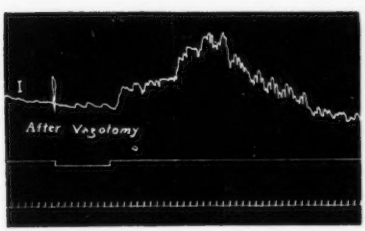

Fig. 6

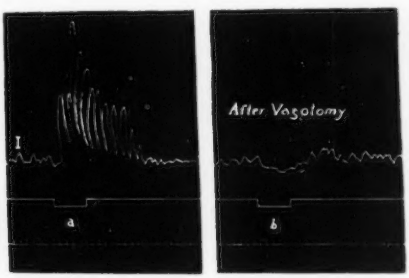

Fig. 7

Fig. 6. Kymograph tracing showing the delayed excitatory response of the small intestine (I) after both vagi have been sectioned. Time in 6 seconds.

Fig. 7. Kymograph tracings showing the immediate excitatory response of the small intestine (I) in the tracing (*a*) which was abolished after vagotomy (*b*). Time in 6 seconds.

observed (fig. 7). The tone of the intestine and colon was raised promptly with increased movement which lasted for the period of stimulus or considerably longer; the latency never exceeded 7 seconds and it returned to the normal quiescent state and tone promptly. This was easily repeatable and no period of rest was found to be necessary. These responses were not intermingled with the delayed response in each individual experiment. In the same animal this immediate response was obtained from points situated dorsally or caudally to the reactive region for the delayed response. However, as often only a limited area was stimulated in each experiment, there was usually only one type of response observed. In rare cases, a combined response of immediate and then delayed excitation with a short interval of quiescence was encountered. These two types of responses, even when they occurred together, could be differentiated without dif-

ficulty. In 2 cats the mammillary body itself was stimulated and no response was observed. Vagotomy in 3 of these animals (table 1) would invariably abolish the immediate response of the small intestine (fig. 7).

In 4 cats, after a delayed excitatory response was demonstrated, the spinal cord was cut at the upper thoracic segment. Stimulation of the same region did not again bring about similar response. Seven chronic cervical spinal cats were stimulated, 3 of which definitely showed no delayed response. In 2 of these animals an immediate excitatory response was observed on stimulating the hypothalamus at the infundibular level, which disappeared after vagotomy. In the 4 others, only a slight but delayed increase of tone and motility of the intestine or colon was demonstrated. In one of these animals, vagotomy was done and the slight response remained unobscured.

TABLE 1

Different types of gastro-intestinal responses following stimulation of the hypothalamus

LEVEL STIMULATED (NUMBER REFERS TO FIGURES)	TOTAL NUMBER OF ANIMALS	NUMBER OF ANIMALS SHOWING: Delayed excitation following unchanged activity (a), inhibition (b) or excitation (c) during stimulus			Only excitation during stimulus	Negative result
		(a)	(b)	(c)		
1	12	3	3	0	1	5
2	15	9	3	1*	0	2
3	16	5	4	0	2†	5
4	10	2	0	0	4	4
Pituitrin (4 pressor units)	5	2	1	0	0	2

* Section of both vagi abolished the immediate excitatory component of the response.

† Section of both vagi abolished the responses.

Discussion. It is easy to understand the mechanism of this immediate excitatory response. Since its onset is abrupt and prompt and its effect lasts as long as the stimulus or only slightly longer, a neural mechanism is definitely suggested. The fact that it is also found in spinal cats and disappears after vagotomy clearly indicates a vagal effect. By and large, this is very similar to the acute response reported by Beattie (1932a) on the stomach and by Masserman and Haertig (1938) on the small intestine; the former found that the gastric effect is abolished after section of both vagi.

As to the delayed excitatory response, its mechanism appears to us to be different. It has a latent period of 40 to 60 seconds, its onset is gradual and its effect lasts for several minutes after the cessation of the stimulus. The responses on the stomach obtained by Beattie and Sheehan (1934) and

Heslop (1938b) were all delayed. The former stated: "There was a latent period of approximately 30 seconds between the commencement of stimulation and the first rise in intragastric pressure. With direct vagal stimulation the latent period was only a few seconds (never more than 10 seconds)." These authors regard all the excitatory responses as vagal. Since our responses were obtained in several vagotomized cats, we cannot suggest that these effects are identical. However, it is to be pointed out that the central effect differs distinctly in many ways from the peripheral vagal effect (figs. 3 and 1, Beattie and Sheehan, 1934), and the difference is strikingly similar to that shown in figure 5 and figure 7 presented here. Furthermore, Heslop in his work on gastric secretion (1938a) reported that anterior hypothalamic stimulation remained successful in accelerating the flow of gastric juice and raising the acidity in 2 chronic vagotomized cats. However, his suggestion that these vagal impulses are reaching the stomach via an alternative route, needs some additional experimental evidence.

It is known that secretion of the posterior lobe of the pituitary gland may be liberated following stimulation of the hypophyseal stalk (Haterius and Ferguson, 1938) and that of the anterior hypothalamus (Clark and Wang, 1939). Though the effect of various fractions of the posterior lobe secretion on the gut is reported to be conflicting (Geiling, 1926) and inconstant (van Dyke, 1936), our own results seem to indicate that it has an excitatory effect on the small intestine and colon (table 1).

On the other hand, in 7 healthy chronic spinal cats, none showed a definite delayed excitatory response on stimulation of the anterior hypothalamus, though in 4 of them (one vagotomized) its presence is suggestive. It is true that many of our stimulation experiments on the normal cats yielded negative results. The absence of a single convincing positive response, however, would certainly make us hesitant in suggesting that this delayed excitation is related to the hypophysis. At the same time, the absence of a similar response in these spinal, but vagus-intact cats would show that this response is not a simple vagal effect. Until further evidence as to its mechanism is available, we deem it wise not to assume that the delayed excitation is effected through the "parasympathetic center" in the anterior hypothalamus (Beattie, 1932b).

SUMMARY

The hypothalamus of more than 50 fasting cats was stimulated by faradic current under light ether or chloralose anesthesia. Its effect on the gastro-intestinal tract was observed through a transparent window or recorded by the balloon method.

Stimulation of the hypothalamus anterior to the infundibular region yielded immediate blanching and occasional inhibition, followed by a

marked excitatory response. Its onset was slow and gradual, and its effect lasted for several minutes. Section of both vagi did not abolish this effect. Its possible mechanism is discussed.

Vagal effects on the gut were obtained when the hypothalamus at or behind the infundibular level was stimulated. Such responses could be elicited in chronic spinal cats and were abolished after bilateral vagotomy.

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THE DISTRIBUTION OF GLUCOSE BETWEEN CELLS AND SERUM: FURTHER EXPERIMENTS WITH HIGH CONCENTRATIONS OF GLUCOSE[1]

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In experiments previously reported (5), in which solutions of glucose were added to blood, it was found that when the concentration of glucose in the diluted blood was less than 1500 mgm. per cent, the glucose became evenly distributed between the water of cells and serum, and the added water was partitioned between the two phases in the same proportion as water added without glucose, or as solutions of urea. The increase in the volume of the corpuscular water was immediate, and almost proportionally equal to that of the water of the serum. When solutions containing higher concentrations of glucose were employed the cells swelled more slowly and when equilibrium had apparently been attained the increment of water in the cells was proportionally much less than the increment in the serum. Furthermore, with such solutions hemolysis did not occur. Since it was found that osmotically significant amounts of base did not escape from the cells, it was inferred that the permeability of the cell membrane was altered in some manner so as to limit the entrance of glucose into the cells.

When a drop of blood was added to 3 cc. of glucose solutions of varying concentrations, hemolysis occurred in those in which the concentration was less than 2300 mgm. per cent. This variation in the concentration of glucose which prevented the free penetration of glucose into the cell indicated that the alteration in permeability is not entirely due to the increase in the glucose concentration. It is, nevertheless, intimately associated with it, since there was no variation when solutions containing low concentrations of glucose were osmotically fortified by the addition of non-electrolytes (sucrose and lactose) and electrolytes to which the cell membranes are impervious. Eisenman, Hald and Peters (1) have shown that in the circulating blood, under conditions possibly associated with metabolic activities of the cells, the base in the cells may change without affecting the distribution of water between cells and serum. The latter is apparently

[1] Aided by a grant from the Fluid Research Fund of Yale University School of Medicine.

controlled by the osmotic pressure of the serum, in which electrolytes are the chief osmotically active components. Similar paradoxical phenomena were not encountered when blood was properly handled *in vitro*. Halpern (4) has demonstrated also that if blood is kept at or below room temperature cellular metabolism of glucose and associated transfers of phosphate proceed too slowly to be significant in the intervals of time involved in the experiments with glucose under discussion.

To define more exactly and possibly to explain the anomalous behavior of blood cells to glucose the experiments here reported were carried out. It was also hoped that they might throw some light on the change in the response of the tissues to glucose when the concentration of this substance in the blood becomes extremely high. The experiments of Wierzuchowski (8) would suggest that when the blood sugar exceeds a certain maximum figure glucose ceases to enter the tissues.

Procedure. To a portion of human venous blood which had been defibrinated by stirring with a glass rod, glucose solution, in concentrations and amounts indicated in columns 2 and 3 of table 1, was added. After the diluted blood, thoroughly mixed, had stood at room temperature for from 15 to 30 minutes, the cell volume of the blood and the concentrations of glucose in whole blood and serum were determined. In most instances these determinations were repeated after an interval of 3 to 5 hours; in 3 instances the final determinations were made after intervals of 26, 48 and 50 hours respectively. In these cases, the blood was kept most of the time in a refrigerator at 5°C. The cell volume of the original was also measured. With the assumption that 72 per cent of the volume of the cells and 93.5 per cent of the serum were composed of water, the concentrations of glucose in the water of the cells and the serum were estimated. In another series of similar experiments, blood and serum were analyzed for inorganic phosphate instead of for glucose. In 2 experiments, in which dry glucose, instead of glucose solution, was added to blood, the measurements of cell volume and glucose concentration were repeated at 5 and 8 hour intervals. In some experiments water, instead of glucose, was added, and the cell volume, serum proteins and base, cell volume and dry weight, or just cell volume determined.

Analytical methods. Glucose determinations were made on a Somogyi (7) filtrate after previously diluting the whole blood or serum to a convenient degree. The Shaffer-Hartman-Somogyi (6) titration method was used.

The Fiske and Subbarow (2) method was used for the determination of inorganic phosphorus. A micro modification devised by Kydd and employed in this laboratory for many years was found quite satisfactory. One-half cubic centimeter of blood or serum was precipitated with 3 cc. of 10 per cent trichloroacetic acid, and to 1 cc. of the filtrate, 0.7 cc. of

water, 0.2 cc. 2.5 per cent ammonium molybdate in acid, and 0.1 cc. sulfonic acid reagent were added. Duplicates were analyzed in all cases.

Serum proteins were determined by the macro-Kjeldahl method.

Methods described by Hald (4) were used in the estimation of sodium and total base of serum.

Cell volumes were measured in a Daland hematocrit tube centrifuged at 1500 r.p.m. for one hour.

Calculations. The increases of cell and serum water were calculated as previously described (5). Estimation of total osmolar equivalents is discussed below.

Results and discussion. The results of the experiments in which glucose was estimated are presented in table 1.

As in previous experiments (5), when glucose solutions were added to blood so that the concentration of glucose was less than 1500 mgm. per cent, the added water and the glucose distributed themselves immediately and almost equally between the cells and serum. With higher concentrations of glucose water entered the cells quite slowly and hemolysis was never induced. Analyses revealed that with the less concentrated solutions the concentrations of glucose per unit of water in cells and serum were approximately equal within 30 minutes. With the more concentrated solutions there was decided inequality in the distribution of glucose, with a tendency to approach equality as the water in the cells gradually increased. When dry glucose was added to blood this approach to equality was accelerated (expts. 17, 17A, 18, 18A and 18B). Although inequality of distribution was noted with a whole blood sugar concentration of 1900 mgm. per cent, in other experiments the glucose of cell water reached 5970 and 6700 mgm. per cent. There is not, then, a limit to the amount of glucose which can be made to enter the cells, but rather a change in the rate at which it enters.

In part the differences in the reactions to weak and strong glucose solutions seem to depend upon the relative speeds with which glucose and water diffuse across the membrane. This is apparent from the fact that as the concentration of glucose is increased, initial contraction of the cells is noted. The failure to attain final equality of distribution might on this account be ascribed merely to the fact that the periods of observation were not sufficiently prolonged. There are, however, arguments against this explanation. First and foremost is the fact that cells cannot be hemolyzed by these strong solutions and, therefore, presumably will not admit unlimited amounts of glucose and water. In the few experiments in which analyses were made as much as 26 and 50 hours after the addition of the glucose solution, the rate of entrance of glucose into the cells is less at the later times than shortly after adding the glucose, indicating, perhaps, that the rate is approaching zero. In addition, when the experiments with dry

TABLE 1

Results obtained when glucose in solution and dry was added to whole blood

The calculations are described in the text

EXPERIMENT NUMBER	SOLUTION ADDED TO BLOOD		INCREASE OF WATER IN		GLUCOSE OF WATER OF		RELATIVE OSMOLAR CONCENTRATION*		TIME AFTER ADDING GLUCOSE
	Volume per cent	Glucose	Cells	Serum	Cells	Serum	Cells	Serum	
	cc.	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*hours*
1	30	3	33	37	920	1030	91	92	0.25
2	40	3	42	52	1030	1212	89	88	0.25
3	20	11	4	31	1295	2480	122	129	0.25
4	40	11	−8	80	1530	4470	138	137	0.25
5	50	11	2	91	2300	4960	142	147	0.25
6	50	11	−1	108	2160	5120	142	146	0.50
7	50	11	−13	106	1650	4880	146	142	0.25
8	60	11	−8	115	1560	5400	141	149	0.25
9	62.5	11	−8	134	2130	5600	136	148	0.25
10	66.7	11	−8	131	1900	5700	145	152	0.25
11	66.7	15	−18	140	2980	7600	182	186	0.50
12	70	15	−29	158	1600	7900	177	189	0.25
13	75	15	−17	139	3300	7820	186	192	0.50
14	83.2	15	−23	164	3100	8600	192	202	0.25
15	70	15	25	115	5600	7350	185	183	2
6A	50	11	26	86	3170	4880	140	146	3
7A	50	11	29	77	3450	4400	139	141	3.25
9A	62.5	11	27	108	3650	5200	149	146	4.75
10A	66.7	11	27	112	3400	5400	143	150	5.50
11A	66.7	15	12	122	4930	7400	185	185	5
16	70	15	3	130	4650	7600	179	187	6
13A	75	15	8	127	4900	7690	190	191	5.25
14A	83.2	15	−13	162	4400	8600	204	200	4.25
12A	70	15	0	135	4200	7680	178	187	9
12B	70	15	11	132	4750	7400	184	182	26
16A	70	15	17	125	4800	7550	174	187	31
16B	70	15	24	121	5440	7380	170	185	48
15A	70	15	37	108	5970	7240	184	185	50
17	Dry glucose added		−21	16	3980	6000	200	196	0.33
18	Dry glucose added		−25	30	5000	8250	220	227	0.50
17A	Dry glucose added		−2	7	5050	5500	195	193	4.50
18A	Dry glucose added		−10	18	6300	7600	227	223	5
18B	Dry glucose added		0	10	6700	7300	222	226	9.25

* Osmolar concentrations are expressed in per cent of the original osmolar concentrations, with the assumption that the latter were equivalent to a 5.50 per cent solution of glucose (for details see text).

glucose are compared with those in which glucose solutions were added, it will be seen that in the former glucose and water are almost equally distributed, while in the latter there is a large discrepancy, although the con-

centrations of glucose in the dry glucose experiments are as high or higher than they are in the experiments with glucose solutions.[2]

At any concentration of glucose in the added solution the amounts of glucose and water that enter the cells seem to be, to some extent, independent of the degree of dilution. This is especially evident in the late observations on the 11.0 per cent groups (expts. 7A and 10A). The cells increase 29 and 27 per cent with cellular glucose concentrations of 3450 and 3400 mgm. per cent in experiments in which the blood was diluted 50 and 66.7 per cent. In dilute solutions the increase of cell volume is directly related to the degree of dilution. Standing alone, this could be taken as direct evidence that the permeability of the cell membrane to glucose is the limiting factor. But the dry glucose experiments, if considered alone, would appear to indicate that the permeability to glucose is not an important factor, and that, possibly, the permeability to water is.

In this connection the previously reported (5) slight differences in the increments of cellular and serum water following the addition of water,

[2] Other experiments were designed to test the possibility that the inequality of glucose distribution was merely a function of slow diffusibility of glucose in high concentration. Blood hemolyzed with saponin was placed in one chamber of a Lavietes filter, serum in the other, and glucose, in solution or dry, added to either side. No pressure was exerted on the intervening cellophane membrane. It was found that glucose in high concentrations diffused very slowly, and more slowly in hemolyzed blood than in serum or in saline. For example, in one set of experiments glucose solution was added to the hemolyzed blood in one filter, to the serum in a second, and in the third serum plus glucose solution was separated by a membrane from serum. The amounts added were about the same. At the end of 8 hours at room temperature, the hemolyzed blood in the first filter contained, per unit of water, more than twice as much glucose as its opposing serum, 6300 mgm. per cent compared with 3000 mgm. per cent; the blood in the second contained less than half as much as its opposing serum, 3250 mgm. per cent compared with 7300 mgm. per cent; but the glucose had diffused with considerable more speed in the filter containing only serum, for the serum which had contained none, now contained 4200 mgm. per cent glucose per unit of water, compared with 5700 mgm. per cent on the other side of the cellophane membrane. At these concentrations equality of distribution in serum and whole blood was not attained in 72 hours, but was in one experiment lasting 96 hours. If both sides of the filter contained serum, glucose distribution was still unequal at the end of 72 hours, but the disparity was not nearly so great as when one chamber of the filter contained hemolyzed blood. When saline was used instead of hemolyzed blood, distribution was almost equal in 24 hours. At lower concentrations, 1500 mgm. per cent, there was equal distribution in water of hemolyzed blood and serum in 8 hours. No significant difference was observed between glucose added in solution and glucose added dry. Although the absence of this difference might invalidate these experiments as being directly analogous, the slow rate of diffusion cannot be disregarded. On the other hand, the comparatively large surface area of the cells in whole blood should certainly facilitate diffusion, compared with the rather small area of cellophane in the filters. Until a more comparable set of conditions can be devised, the factor of the rate of diffusion of glucose cannot be evaluated.

urea solution and glycerol solution to blood become pertinent. The addition of water to whole blood was repeated in a number of experiments and the percentage dilutions of cells and serum were calculated from the observed cell volumes, the water contents of whole blood and serum, the serum protein concentrations, or from serum base concentrations. The results are presented in figure 1, and completely confirm the previous

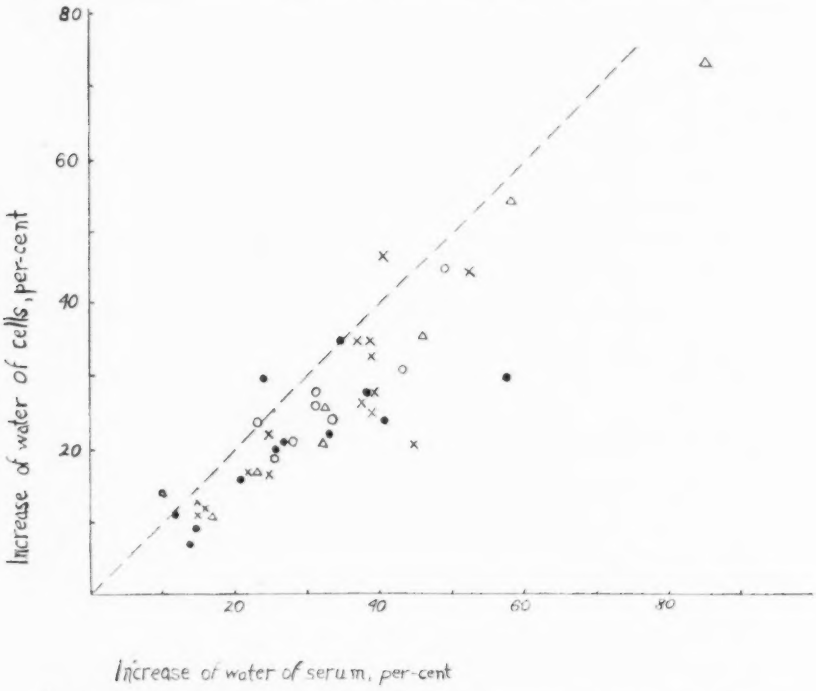

Fig. 1. Percentage increases in water of cells and serum when water was added to blood. Calculations were made from cell volume, ●, from cell volumes and determined water contents, ×, and from cell volumes, serum proteins and serum bases, ○. Certain of the experiments, △, have been presented in a previous publication (5). The broken line is placed at an angle of 45°.

observations. The failure of the cellular water to increase as much, proportionally, as the serum water is quite definite, and is associated with an apparent osmotic anomaly not found when solutions of electrolytes are added to blood *in vitro* (1), for the ratio (water of $cells_2$/water of $cells_1$: (base of serum H_2O_1/base of serum H_2O_2)) is not unity, but closer to 0.95.

Because, however, of experiments already cited, and because of the ease with which hemolysis is induced with dilute glucose solutions, and es-

pecially since cells at first shrink in strong glucose solutions, it is hardly permissible to assign the apparently changed permeability merely to the addition of water. For the present it must be surmised that both glucose and water control the penetration of glucose into the cell, and that to neither alone can be assigned a position of prime importance in an interpretation of the results.

With the concentrations of glucose in water of cells and serum known, and since electrolytes do not cross the cell membrane, the osmolar concentrations of the two phases can be compared at any point in the experiments. Serum and cells are isotonic with a 5.5 per cent solution of glucose, and therefore presumably contain the same osmolar equivalents as such a solution. If it be assumed, then, that cells and serum originally contained an osmolar concentration of 100 per cent or 5500 mgm. per cent of glucose, and due correction be made for changes in volume of the two media, the total osmolar concentration of the final contents of cells and serum can be estimated by merely adding the analytically determined concentrations of glucose in comparable terms of osmolar equivalents. The experiments with water added would indicate the possibility that the electrolytes in the cell change their osmotic activities, but this possibility should offer no insurmountable objection, since such a change would almost certainly be slight, and so affect little the results of the calculations, which are only approximations. It is evident from these estimations (table 1, columns 8 and 9) that at every stage of the transfers of water and glucose osmotic equilibrium is maintained, even when the distribution of glucose is most disparate.

Analyses for inorganic phosphorus revealed no changes that could not be attributed merely to changes in the relative volumes of cells and serum. The passage of glucose across the cell membrane, therefore, involved no demonstrable reactions with phosphorus such as are noted when metabolism of carbohydrate in the cells is active.

Such unequal distribution of glucose has been advanced as evidence that there is "bound water" in cells, that is, water that is not available as solvent. Dilution of blood with water seems to strengthen this concept. Nevertheless, although no alternative explanation for the anomalous behaviors of glucose and water has been found, the weight of accumulated evidence is against such a concept, and the phenomena described are quite incompatible with it. The estimations of osmotic pressure prove that within the limits of error of the methods employed, all the water in cells and serum is available for the solution of solutes in general. In dilute glucose solutions there is complete equality of glucose distribution. In strong solutions the degree of inequality varies with the concentration of glucose solution added and the lapse of time. That factors other than these are involved, however, is evident from the fact that while the addi-

tion to blood of 15 per cent glucose solution results in a greater inequality than does the addition of 11 per cent glucose, the inequality of distribution consequent on the addition of dry glucose is much less than that observed with either of these solutions.

SUMMARY

When concentrated solutions of glucose are added to blood there is a marked discrepancy between the amount of glucose taken up by the cells and that remaining in the serum. The disproportion tends to diminish with time, but does not disappear completely. The limiting factors in the free penetrability of the cell membrane to glucose are discussed but are not precisely defined. It is demonstrated, however, that despite the discrepancy in the distribution of glucose, osmolar equality between cells and serum is still maintained.

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THE RELATIONSHIP BETWEEN DIFFERENTIAL PRESSURE AND BLOOD FLOW IN A CORONARY ARTERY[1]

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In 1934 Wiggers (1) proposed a procedure which with certain modifications was used to study the phasic variations of flow in all three coronary arteries (2, 3, 4). This procedure involved the subtraction of the peripheral coronary arterial pressure from the central coronary pressure. It yielded a curve (differential pressure curve) whose ordinate values were assumed to be at each instant proportional to the rate of coronary flow.

The present communication is concerned with an experimental investigation of this assumption. For this purpose a procedure was devised, including the development of a new type of flow meter, by which it is possible to register both the peripheral coronary pressure and the phasic changes of inflow into the coronary artery at predetermined heads of pressure. By comparing the rate of inflow with the corresponding differential pressure (difference between perfusion pressure and peripheral coronary pressure) the relationship between differential pressure and flow has been determined.

METHODS. *Apparatus.* The flow meter (see fig. 1) consists of a reservoir, *B*, containing about 5 cc. of blood, which connects through a three-way stopcock, *S*, and lead tube with the artery to be perfused. The reservoir is expanded at its upper end into a chamber, *A*, filled with air at the pressure at which it is desired to perfuse the artery. A sensitive optical manometer, *MF*, incorporated as an integral part of the chamber records the slight decline of the air pressure as the blood leaves the reservoir, the rate of decline of pressure indicating the perfusion rate. The meter is rigidly supported by a heavy bar fastened to *M*.

The peripheral coronary pressure and perfusion pressure are recorded by a Gregg pressure manometer, *MP*, connected with the lead tube. Both manometers, *MF* and *MP*, are fitted with special rubber membranes,

[1] Preliminary reports were made before the Cleveland Section of the Society for Experimental Biology and Medicine, March, 1939, and before the American Physiological Society, Toronto, Canada, April 27, 1939.

that of the flow meter being 0.006 inch thick and that of the pressure manometer 0.020 inch thick, and supplied with +1.0 dioptre planoconvex mirrors. At a projection distance of 4 meters the former has a sensitivity of 20 mm. for 1 mm. Hg with a natural frequency of 150 per second and the latter a sensitivity of 40 to 60 mm. for 100 mm. Hg with a natural frequency of 200 to 250 per second.

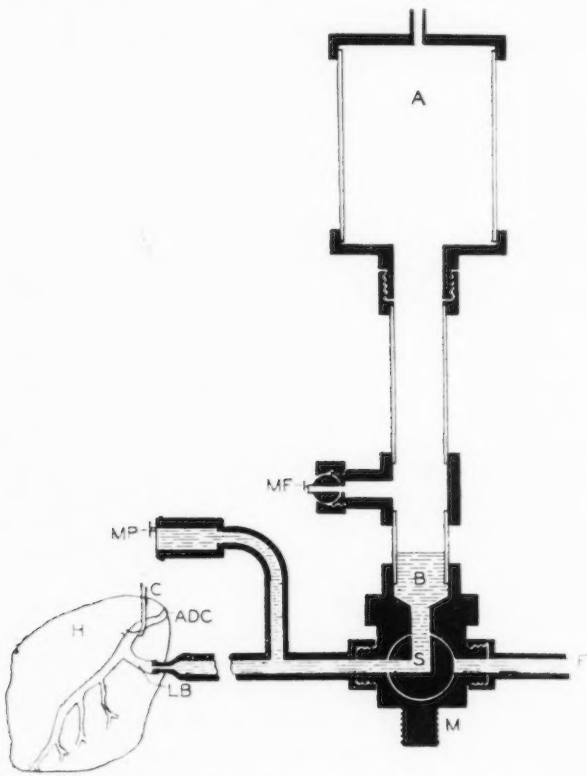

Fig. 1. Diagram of flow meter. Description in text

EXPERIMENTAL PROCEDURE. Successful experiments were performed on eight dogs with essentially the same results. After anesthetization with morphine and sodium barbital and institution of artificial respiration the heart was exposed and a short length of the ramus descendens together with a suitable side branch dissected free. The animal's blood was rendered noncoagulable with heparin (75 units per kgm.) and chorazol fast pink (80 mgm. per kgm.). The side branch of the coronary was cannulated

and connected with the flow meter as shown in figure 1 and another pressure manometer similar to the one described above was connected with the aorta.

Operation of apparatus. The reservoir is partially filled with blood (see fig. 1) and the pressure in the chamber is raised to the desired level (20 to 200 mm. Hg). After recording a few heart beats the main coronary vessel is clamped (at *C*, fig. 1), and the stopcock in the flow meter turned to the position shown in figure 1. As the blood enters the artery the chamber pressure drops slightly resulting in a progressive movement of the flow meter and coronary pressure beams (from left to right) across the photokymograph lens. By varying the size of the chamber its rate of pressure drop can be generally kept below 1 mm. Hg per heart beat and the sensitivity so controlled that the flow beam moves 12 cm. (the width of the sensitive paper) in 3–5 heart beats.

To obtain the data necessary for constructing the differential pressure curve for comparison with the flow determinations the stopcock (*S*, fig. 1) is rotated 90° to the right, the coronary artery is clamped for 8–10 beats and the peripheral coronary pressure recorded with the pressure manometer (*MP*, fig. 1). The correct systolic value of the peripheral coronary pressure is recorded by intermittently clamping the coronary artery during systole at a rate slightly asynchronous with the heart rate. (See reference (3) for details.)

The flow meter is calibrated during an experiment by recording the amplitude of the deflection at a series of different perfusion pressures when 0.5 cc. of fluid is driven into or out of the reservoir with a tuberculin syringe.

Adequacy of the meter. The meter was tested as follows: 1. the meter was filled and connected by lead tubing to a 10 cc. syringe, the plunger of which (activated by a mechanical device) moved in and out of its barrel with a sine wave motion and caused fluid to enter and leave the meter. The plunger movements were recorded simultaneously with the flow meter beam. In figure 2A with the plunger, *P*, making 12.5 double strokes per second (stroke volume 1 cc., *M*) the lag of the meter is less than 0.005 second while at lesser frequencies the lag is too small to read. 2. The flow from the meter (at various air chamber pressures) was started and stopped abruptly either by rotation of stopcock, *S*, or by an intermittent compression of a rubber tube by an electromagnetic clamp (fig. 2B). There is no evidence of appreciable lag or overshooting.

Results. In segment A of figure 3 the aortic pressure, *AP*, and coronary pressure, *CP*, are recorded simultaneously.[2] At time *K* the central coronary artery was clamped and the coronary manometer began to

[2] To facilitate inspection vertical lines A, B, C, D, A^1 have been drawn through simultaneous points on the curves.

record the pressure in the coronary artery distal to the clamp. At point S the stopcock S (fig. 1) was turned so that blood under pressure of 113 mm. Hg (slightly less than aortic systolic) entered the artery from the reservoir. The coronary artery manometer beam swings up and begins recording

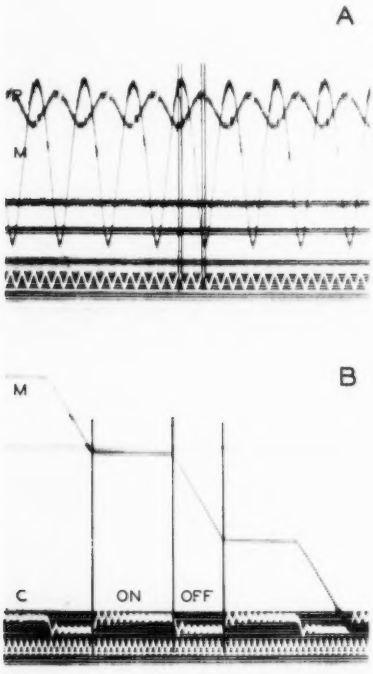

Fig. 2. A. Test of adequacy of flow meter. M, record written by meter, upward movement = inflow of blood into meter; P, record written by a mirror attached to moving plunger of a syringe connected by a lead tube with the meter, downward movement = expulsion of blood from syringe into meter; time, $\frac{1}{5}$ second; lag, indicated by vertical lines = 0.005 second.

B. Test of adequacy of flow meter. Meter outflow interrupted by an electromagnetic clamp arranged to compress a short length of thin walled rubber tube through which fluid leaves meter. M, record written by meter; C, record of signal in series with the clamp, upward movement indicates compression of tube, downward movement release. Time, $\frac{1}{5}$ second.

the perfusion pressure, PP. Within a short interval the flow beam, previously off the field, begins to cross the record in an irregular slanting line, F. A definite flow exists throughout the cardiac cycle.

During systole the rate of flow begins to diminish at A^1 (i.e., at the

beginning of the isometric contraction period), decelerates further to *B* and then remains relatively constant from *B* *C*.

During diastole the rate of flow starts to increase at *C* (coincident with the decline of the peripheral coronary pressure curve) and reaches a maximum at *D*, i.e., at about the diastolic valley of the peripheral coronary

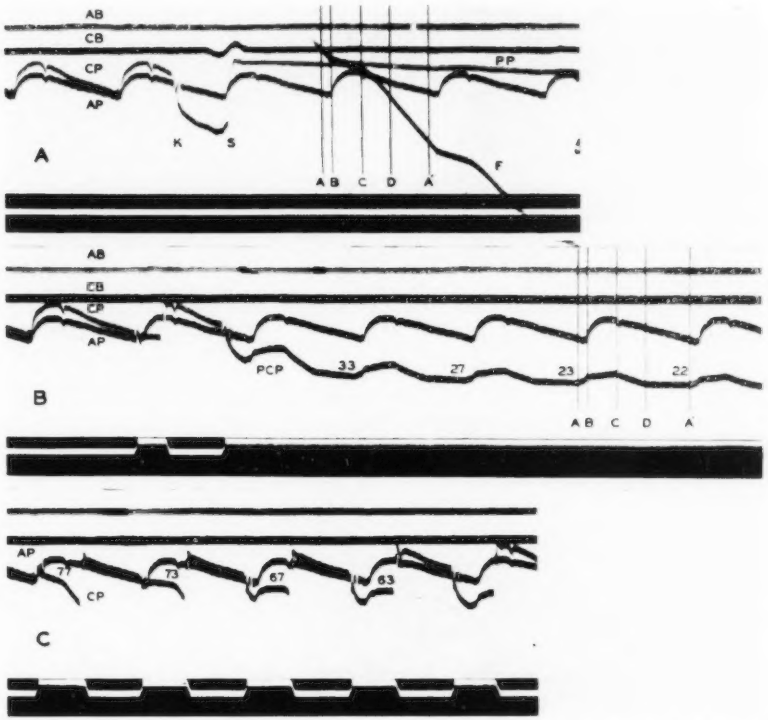

Fig. 3. Comparison of differential pressure with flow. A. Record of flow. B. Determination of diastolic peripheral coronary pressure. C. Determination of systolic peripheral coronary pressure. *AP*, aortic pressure; *CP*, coronary pressure; *AB* and *CB*, base lines for aortic and coronary manometers respectively; *PP*, perfusion pressure, measured with coronary manometer; *K*, time of clamping main coronary vessel; *S*, instant when stopcock (*S*) turned to position shown in figure 1; figures indicate pressures in millimeters of mercury. For other details see text.

pressure curve. In late diastole (*D* A^1) the rate of flow is essentially constant.

Comparison of the recorded flow curve with the flow curve constructed from the differential pressure curve. For this purpose the necessary tracings and reconstructions of figure 4 have been taken from figure 3.

Curve F (solid line), figure 4B, is a tracing of the recorded flow curve in figure 3A. Curve F (dashed line)[3] is the theoretical flow curve reconstructed from the differential pressure curve, DP. The latter differential curve was constructed as follows: The peripheral coronary pressure curve of figure 3B was raised to its proper ordinate value of 67 mm. Hg (cf.

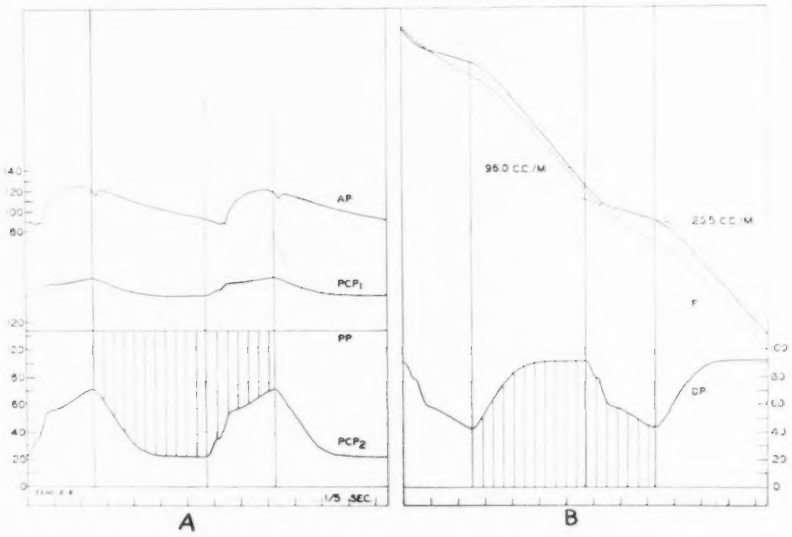

Fig. 4. A. Calculation of differential pressure. AP, aortic pressure, calibration at left; PCP_1, tracing of peripheral coronary pressure curve; PCP_2, peripheral coronary pressure curve (PCP_1) enlarged to correspond to aortic pressure scale, calibration at left; PP, line corresponding to level of pressure at which coronary artery was perfused; vertical lines, differential pressure (perfusion pressure minus peripheral coronary pressure).

B. Construction of theoretical flow curve from differential pressure curve and comparison with recorded flow curve. F, flow curves, solid line = recorded curve, dashed line = constructed curve; DP, differential pressure, from A, scale at right. Theoretical flow curve constructed so that slope at each point is proportional to the corresponding differential pressure, the slope at the end of the first diastole being made equal to that of the recorded curve; figures correspond to rates of flow in latter part of diastole and midsystole respectively, in recorded curve.

fig. 3C) and appears in figure 4A as PCP_2. This was then subtracted from the constant infusion pressure of 117 mm. Hg (PP of fig. 4A). The difference (vertical shading) (fig. 4A, B) represents the moment to moment

[3] Arbitrarily, the reconstructed and recorded flow curves were drawn with the same slope at the end of diastole while the slope at all other points was made proportional to this slope.

differential pressure between the perfusion pressure and the peripheral coronary pressure.

While it is realized that considerable error is involved in such reconstructions still if the differential pressure curve gives a reasonably correct picture of the moment to moment flow then a reconstructed flow curve drawn from it so that the slope at each point is proportional to the differential pressure should approximate the contour of the recorded flow curve. Certain differences are at once obvious in the reconstructed flow curves: 1, the flow during systolic ejection is greater and is more gradually reduced; 2, minimum flow is reached later, i.e., at the peak of the peripheral coronary pressure; 3, the acceleration of flow is less rapid during early diastole. On the other hand, at the instant corresponding to the peak of the peripheral coronary pressure curve the slope of the recorded curve has increased sufficiently to approximate that of the reconstructed curve.

In making these comparisons one assumption made in previous work required experimental validation, i.e., the peripheral coronary pressure curve was considered as that pressure which at each moment would just not cause fluid to flow through intramural vessels. To test the accuracy of this concept for two points on the flow curve, i.e., the maximal and minimal points, the coronary bed was perfused with blood at pressures approximately equal to these two values. The resultant flow curves and *PCP* curves redrawn from the original records are presented in figure 5. At perfusion pressures approximately equal to the diastolic minimal value of the peripheral coronary pressure (12 mm. Hg, cf. lines PP_2, fig. 5) there is a to and fro movement of blood but no forward flow (line F_2). When the perfusion pressure is raised to approximately the systolic maximal value (82 mm. Hg, line PP_1) the diastolic flow is considerable (line F_1) but the systolic flow is practically zero except for the isometric contraction period.

From these findings the conclusion is drawn that the maximal and minimal values of the peripheral coronary pressure curve equal those heads of pressure which will just not cause fluid to flow into the coronary bed during the middle of systole and the latter part of diastole.

The normal relationship between differential pressure and flow. In previous publications the rate of intramural flow at each instant was considered proportional to the differential pressure (head of pressure minus peripheral coronary pressure). Measurements[1] made on 125 records in different experiments showed that the systolic rate of flow per millimeter of mercury differential pressure is consistently less than the diastolic for

[1] The points chosen for measuring the systolic and diastolic flows were the latter portions of *B-C* and *D-A*[1] (fig. 3) during which the peripheral coronary pressure curve is changing minimally. In some records the systolic flow was determined at the peak of the PCP curve.

the same heart cycle (cf. table 1). For example, in figure 4 during systole with a minimal differential pressure of 43 mm. Hg the flow is 25.5 cc. per minute or 0.59 cc. per minute per mm. Hg, while during diastole the flow is 1.04 cc. per minute per mm. Hg.

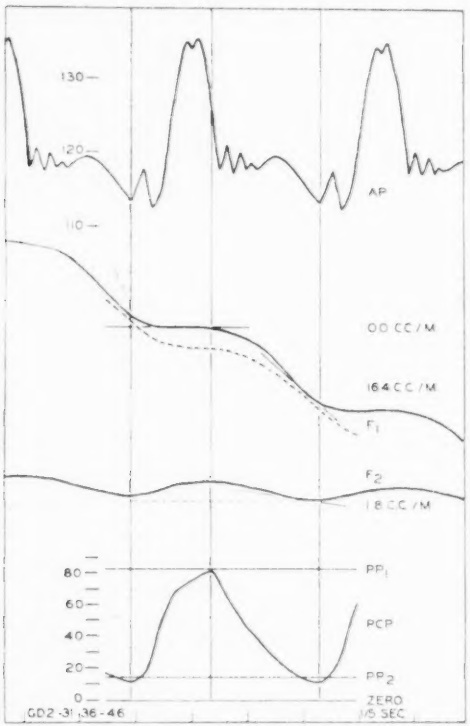

Fig. 5. Tracings of curves to show rates of flow when coronary artery perfused at pressures corresponding to systolic and diastolic levels of peripheral coronary pressure. *AP*, aortic pressure, calibration at left; F_1, flow curve when coronary artery perfused at a pressure equal to the systolic value (PP_1) of the peripheral coronary pressure curve (PCP); F_2, flow curve when coronary artery perfused at a pressure equal to the diastolic value (PP_2) of the peripheral coronary pressure; calibration, lower left, refers to peripheral coronary pressure curve and lines indicating perfusion pressure; figures at right of flow lines indicate the rates of flow at mid-systole and late diastole for the two flow curves.

To study further the relationship between flow and differential pressure, the coronary bed was perfused at a variety of pressures and the rates of flow during the middle of systole and during the latter part of diastole compared with the resulting differential pressures. If the rate of flow

per millimeter of mercury differential pressure were always constant a plot of the values should fall along a straight line passing through the origin. Figure 6 is a graph of the results of a typical experiment. Despite the considerable spread of points, it is obvious that 1, the systolic flow is generally less than the diastolic for a given differential pressure; 2, above 20–30 mm. Hg differential pressure the flow bears a linear relationship to differential pressure during both systole and diastole.

Discussion. The evidence presented in this paper confirms previous observations that the systolic peripheral coronary pressure is generally less than the aortic pressure and that there is a sizable inflow throughout systole when the coronary arteries are perfused with blood at aortic systolic pressure.

TABLE 1

EXPERIMENT	AORTIC PRESSURE			SYSTOLE			DIASTOLE			FLOW, CC. PER MIN. PER MM. Hg, DIFF. PRESSURE	
	Syst.	Diast.	Perf. Press.	P.C.P.	Diff. Press.	Flow	P.C.P.	Diff. Press.	Flow	Syst.	Diast.
						cc. per min.			*cc. per min.*		
1	100	73	90	80	10	9.1	22	68	142	0.91	2.09
	110	85	110	85	25	11.9	22	88	117	0.48	1.35
2	137	95	124	89	35	13.0	35	86	102	0.37	1.19
3	120	85	115	70	45	25.5	20	95	96	0.57	1.01
	150	117	158	78	80	85.0	23	135	186.0	1.04	1.37
4	108	98	110	73	37	2.4	20	90	12.9	0.065	0.143
	122	100	160	80	80	4.4	20	140	18.0	0.055	0.13

The rapid rate of forward flow recorded by the meter during isometric relaxation, exceeding that predicted from the differential pressure curves (fig. 4) and in some instances even exceeding that during the latter part of diastole, probably represents inflow of fluid to fill the larger vessels compressed by the intramural tension during the preceding systole rather than intramural flow. Likewise the retardation of inflow during isometric contraction and midsystole is probably more than the actual slowing of the intramural flow caused by the restriction in the size of the available vascular bed.

The findings presented (see fig. 6) indicate that above a differential pressure of thirty millimeters of mercury there is a fairly linear relationship between differential pressure and coronary inflow for both systole and diastole, but that the inflow during the middle of systole is proportionally less than that during the latter part of diastole. Three possible explanations operating separately or together are advanced to explain the smaller systolic flow: 1, for the same differential pressure the intramural vessels

during systole undergo less expansion with the increased pressure because of their greater extravascular support; 2. the deeper lying vessels are closed during systole thereby reducing the available bed; 3. the potentially

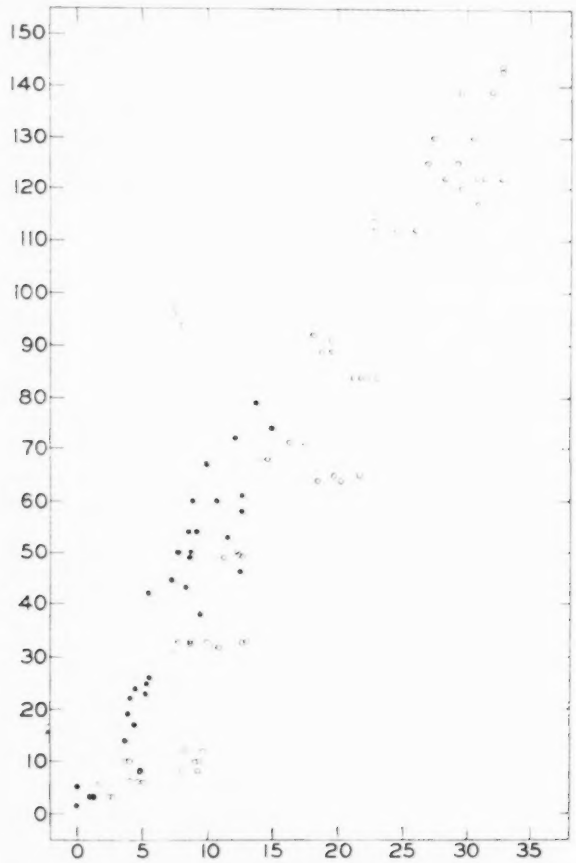

Fig. 6. Plot of the relationship between flow in cubic centimeters per minute (abscissa) and differential pressure in millimeters of mercury (ordinate) for mid-systole and late diastole measured in a large series of records from one experiment. The different differential pressures were obtained by perfusing the artery at various pressures. Circles represent flows in diastole, dots flows in mid-systole.

greater compression force in the deeper layers of the myocardium displaces blood into more superficial vessels.

Since in any one experiment the blood flow and differential pressures vary in the same direction but not always by the same percentage amount,

the method of differential pressures may be used to determine the general contour of the intramural flow but may not be used in a strictly quantitative manner to determine the magnitude of the phasic variations in coronary flow.

SUMMARY

The moment to moment rate of flow into coronary arteries was measured while the vessels were perfused under relatively constant heads of pressure.

Comparison of the rate of blood flow into a coronary artery with the differential pressure (difference between the perfusion pressure and peripheral coronary pressure) shows that: 1, the peripheral coronary pressures indicate the exact time relations of the changes of resistance to flow in the coronary arteries; 2, the systolic and diastolic values of these curves correctly represent the heads of pressure that will just not cause inflow of blood during the respective periods of the cardiac cycle; 3, the differential pressure curves represent the direction and roughly the magnitude of the phasic changes of coronary flow but underestimate the exact value of the moment to moment coronary flow.

The curves obtained indicate that under normal conditions (perfusion of the coronary artery with blood from the aorta) the intramural blood flow will show a sudden retardation with the onset of isometric contraction, during systole forward flow will persist in most hearts, with the onset of isometric relaxation forward flow will rapidly increase and will remain rapid during the latter part of diastole diminishing slightly with the decline of the head of pressure in the aorta.

The authors wish to express their appreciation to Doctors Eckstein, Boyer and Wegria for assistance in the performance of some of the experiments.

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EFFECTS OF VISCOSITY, ISCHEMIA, CARDIAC OUTPUT AND AORTIC PRESSURE ON CORONARY BLOOD FLOW MEASURED UNDER A CONSTANT PERFUSION PRESSURE[1]

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The normal coronary inflow together with the alterations in coronary flow induced by various circulatory changes have been studied by the method of differential pressures (1, 2, 3). With the development of a constant pressure flow meter (4), however, it was found that *for normal flow* the differential curve represents the directional changes in flow but does not indicate the exact value of the moment to moment coronary flow. Therefore we have reëxamined various determinants of flow and have compared the recorded alterations of differential pressures with the actual changes of coronary flow.

The operative exposure and experimental methods for recording differential pressures and flow were identical with those previously described (1, 2, 4). The inflow into the coronary artery was measured by the constant pressure flow meter and compared with the existing differential pressures during appropriate control periods and 1, during perfusion of the coronary bed with Locke's solution (decreased viscosity plus slight anoxia); 2, immediately after a temporary period of ischemia of the coronary bed; 3, after augmentation of the cardiac output by increasing the venous return (transfusion of blood or intravenous infusion of Locke's solution), and 4, at different aortic pressures induced by compression of the lower thoracic aorta.

For comparison of the flows, measurements were made of the systolic and diastolic rates of flow and also of the flow per minute per millimeter of mercury differential pressure. For measuring the flows an interval was chosen in systole and another in diastole at which the peripheral coronary resistance was considered to be relatively constant, i.e., for the former at about the latter third of systole and for the latter late in diastole.

RESULTS. These are set forth in figures 1 and 2 and in table 1. The data under any one letter in the table are taken from the curves of the figures of the same letter.

[1] Preliminary reports of this work were presented before the American Physiological Society at Toronto, Canada, April 27, 1939, and before the Cleveland Section of the Society for Experimental Biology and Medicine, March, 1939.

The effect on coronary flow of substituting Locke's solution for the animal's own blood is illustrated in figure 1A and B. In A, perfusion of the ramus descendens with blood at 115 mm. Hg pressure (slightly under aortic systolic) gives systolic and diastolic rates of flow of 3.15 and 19.4 cc. per minute respectively. After filling the constant pressure meter, cannula and superficial part of the coronary artery with Locke's solution the rates of flow determined at the same infusion pressure are 12.9 cc. in systole and 66 cc. per minute in diastole. The peripheral coronary systolic

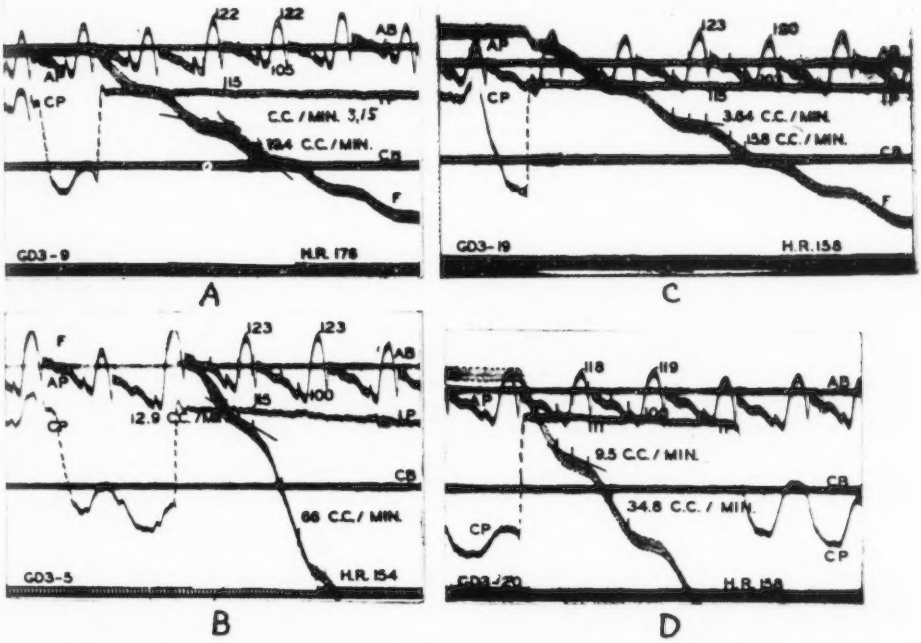

Fig. 1. Records illustrating the effect on coronary blood flow of substitution of Locke's solution for blood (A vs B) and of temporary ischemia of the coronary bed (C vs D). A, B are the respective controls. AP, aortic pressure; CP, coronary pressure; AB, CB, aortic and coronary base lines; IP, infusion pressure measured with coronary manometer; F, coronary inflow.

and diastolic pressures are unchanged in the two conditions (85 vs. 87 mm. Hg for systole and 20 vs. 23 mm. Hg for diastole). Hence with the same differential pressure existing during the two systoles (30 vs. 28 mm. Hg) and diastoles (95 vs. 92 mm. Hg) the corresponding flows are 400 and 300 per cent of the control values.

In other experiments in which the perfusion pressure approximates either the systolic or diastolic peripheral coronary pressure the rate of flow of Locke's, like the controls with blood, approaches zero.

Following temporary ischemia of the coronary bed[2] the inflow is increased greatly without significant alterations of the peripheral coronary pressure. A typical set of records illustrating this point is presented in figure 1C, D. In the control, C, with a peripheral coronary pressure of 90 mm. Hg systolic and 25 mm. Hg diastolic and an infusion pressure of 115 mm. Hg the rates of flow are 3.6 and 15.8 cc. per minute during systole and diastole respectively. After a two minute interruption of the blood supply to the coronary artery the aortic pressure is but slightly lowered and the peripheral coronary pressure is unchanged (D). Despite this the systolic and diastolic flows are increased to 9.5 and 34.8 cc. per minute respectively.

TABLE 1

FIGURE NUMBER	AORTIC PRESSURE			INFUSION PRESSURE	PERIPHERAL CORONARY PRESSURE		DIFFERENTIAL PRESSURE		FLOW, CC. PER MIN.		FLOW DIFF. PRESSURE		CONDITION
	H.R.	Syst.	Diast.		Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	
1A	176	122	105	115	85	20	30	95	3.15	19.4	0.10	0.10	Normal
1B	154	123	100	115	87	23	28	92	12.9	66.0	0.46	0.73	Substitute Locke's for blood
1C	158	123	103	115	90	25	35	90	3.6	15.8	0.14	0.18	Normal
1D	158	119	100	115	90	25	25	90	9.5	34.8	0.38	0.37	Ischemia
2A	172	122	105	116	87	20	29	96	2.94	13.97	1.01	1.45	Normal
2B	156	128	110	116	85	20	31	96	2.95	20.10	0.95	2.09	Increased cardiac output
2C	98	70	41	69	53	18	16	51	4.3	44.5	0.27	0.87	Normal
2D	98	135	90	135	89	35	40	94	27.4	108.0	0.69	1.15	Increased aortic pressure

Increased cardiac output augments total coronary flow during diastole while during systole the effect is somewhat variable. The results of the intravenous infusion of blood on coronary flow are reproduced in figure 2 (B as compared with A). Following slow infusion into the jugular vein the aortic pressure is moderately increased and both the systolic and diastolic values of the peripheral coronary pressure are slightly elevated. Perfusion of the coronary bed with blood at a pressure somewhat less than aortic systolic (115 mm. Hg) during both the control period (A) and that of augmented output (B) increases by approximately 40 per cent the diastolic flow, while the systolic flow is unchanged.

When the aortic pressure is raised by compression of the aorta the pe-

[2] The period of ischemia lasted in different experiments from 30 seconds to 2 minutes, an interval of time sufficiently long that it may be safely assumed that the muscle in the perfused area was extending instead of shortening during systole (5).

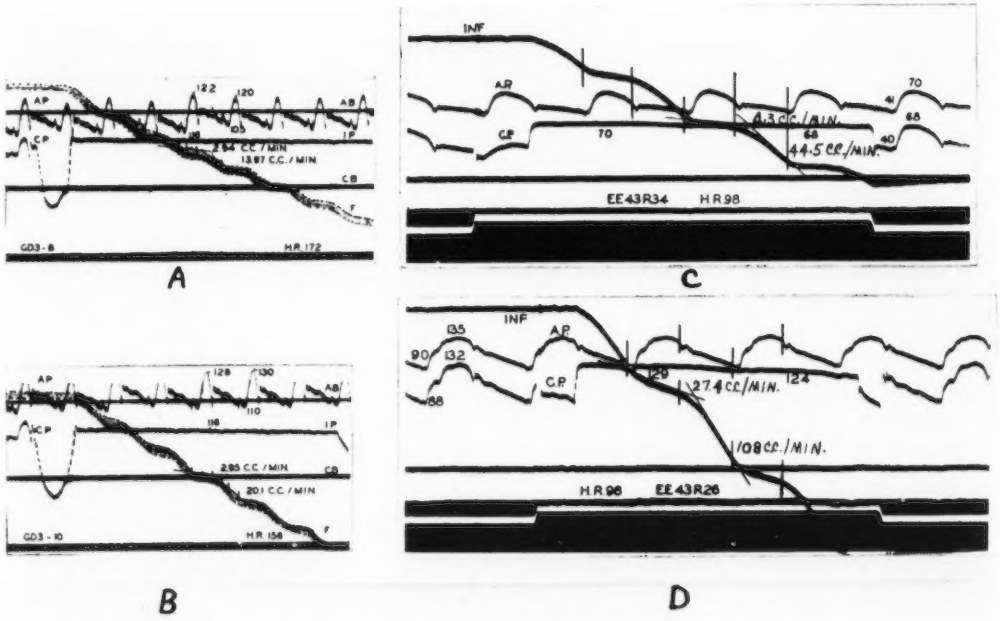

Fig. 2. Records illustrating the effects of intravenous blood infusion (A vs B) and of elevation of aortic blood pressure (C vs D) on coronary inflow. INF, coronary inflow. Other letters same as in fig. 1.

ripheral coronary systolic and diastolic pressures are raised but not as much as the corresponding aortic pressures. The result is a marked increase in both systolic and diastolic differential pressures. This confirms a previous investigation (6). If now the coronary bed is perfused with blood under a pressure corresponding to the aortic systolic the flow is increased throughout the cardiac cycle (fig. 1C, D).

Such comparisons of coronary flow and differential pressures show as observed previously (4) that in the same cycle the systolic flow is generally less than the diastolic for the same differential pressure (cf. table 1). In comparing systoles and diastoles in different circulatory conditions, the flows may or may not change in the same direction as the differential pressures. Following elevation of aortic pressure the flows and differential pressures both increase while in the other conditions listed here the flows all increase but the differential pressures either decrease or undergo no significant change. In addition, the magnitude of the alterations of flow bears no set relationship to the shifts of differential pressure; as a rule the ratio of flow to differential pressure increases greatly (except during systole of increased cardiac output in which the ratio decreases slightly).

SUMMARY AND CONCLUSIONS

The effects of several altered circulatory conditions on coronary flow have been studied during perfusion of the coronary artery with blood under a constant head of pressure. We have confirmed previous findings (using the method of differential pressures) that in elevation of blood pressure and increased cardiac output following augmented venous return, the coronary bed receives an increased blood supply because, although the aortic pressure rises, the peripheral coronary pressure fails to rise as much. However the increase of flow which actually occurs is generally somewhat greater than that predicted from the differential pressures.

In addition, the reduction in viscosity of the perfusate by substitution of Locke's solution for blood causes an unexpectedly large increase of flow, amounting at times to 300–400 per cent of the rate observed with blood. Also, a period of ischemia of the coronary bed greatly increases the flow during the initial period of restored circulation. Such flow augmentations are suggested by the concomitant slight decrease in peripheral coronary pressure, but the method of differential pressures fails to indicate the magnitude of the flow change.

It is concluded that peripheral coronary pressure curves can accurately represent the time relations of the change of flow to the aortic pressure variations but do not indicate the magnitude of the change in resistance to flow under various circulatory conditions; hence the flow itself is underestimated.

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REGISTRATION AND INTERPRETATION OF NORMAL PHASIC INFLOW INTO A LEFT CORONARY ARTERY BY AN IMPROVED DIFFERENTIAL MANOMETRIC METHOD[1]

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Phasic coronary flow and its determinants have been studied by the method of differential pressures (1, 2, 3, 4) and by the constant pressure flow meter (5, 6). Both are laborious and in addition the former may at times underestimate the extent of variations of flow while the latter does not evaluate the effect of the pulsation of the central coronary pressure.

To obviate these difficulties a method has been devised in which blood from a branch of the aorta is directed through a short external circuit containing a differential meter and then into a coronary artery. This paper is concerned with the use of this meter in registering both the moment to moment rate of inflow and the total inflow into the coronary arteries of dogs under essentially normal conditions.

Apparatus. The meter consists of a device which generates a difference of pressure (roughly proportional to the rate of flow) between two points, and a differential manometer for recording this difference of pressure.

Blood flowing from the aorta *via* the subclavian artery (*SC*, fig. 1) to the coronary artery, *SBC*, passes through a metal tube 2.39 mm. in diameter, approximately 4 cm.[2] long and containing a very thin orifice plate, *D*. The size of the orifice is varied to control the magnitude of the difference of pressure and therefore the sensitivity of the meter. An orifice about 1.27 mm. in diameter was usually employed. Two side tubes (*UT* and *DT*) each 1.19 mm. in diameter open off the main tube. The centers of these are 1.19 mm. from the orifice. To facilitate cleaning and substitution of orifice plates of different sizes the main tube is constructed in two parts which in use are held firmly together by a shell and screw cap (*S* and *S*). In some experiments a Pitot tube arrangement has been substituted for the orifice.

[1] Preliminary reports of this work were presented before the American Physiological Society at the Toronto Meeting, 1939, and before the Cleveland Section of the Society for Experimental Biology and Medicine, May, 1939.

[2] The actual dimensions may be varied at will but the same proportional relationship should be retained.

When flow occurs, the blood is momentarily accelerated as it passes through the orifice and retains this acceleration for several millimeters downstream. As a result, the velocity of flow past the downstream side tube, *DT*, is greater and therefore its lateral pressure is less than that of the fluid flowing past the upstream lateral tube, *UT*. This pressure difference becomes greater the more rapidly the fluid flows.

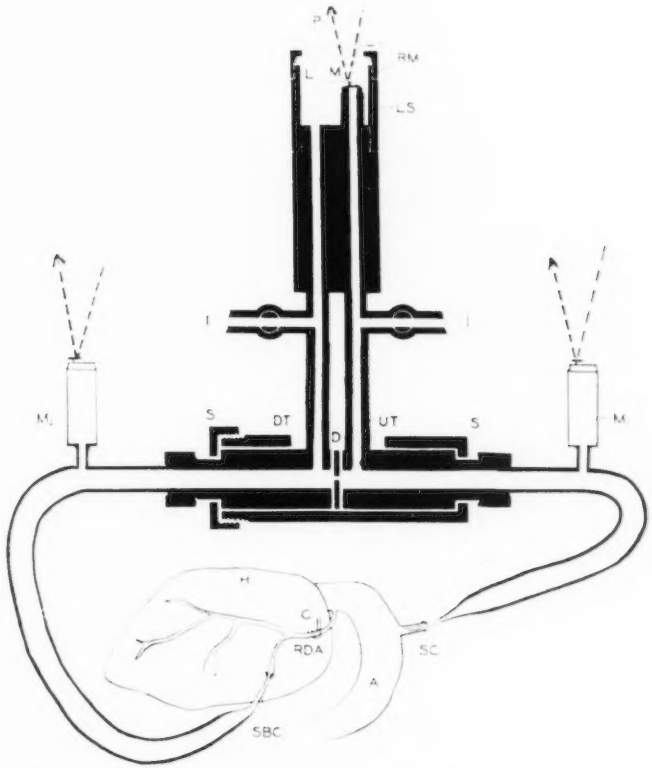


Fig. 1. Diagram of orifice, differential manometer and connections for measuring coronary inflow. See text for discussion and description.

The two lateral pressures are conducted by lead tubes to the differential manometer which is similar in principle to but different in design from that devised by O. Frank (7). The upstream lateral pressure is transmitted to one side of a special rubber membrane, *RM*, 0.003 to 0.006 inch thick stretched 3–5 times over a manometer tip 4 mm. in diameter. The downstream lateral pressure is transmitted to the other side of this membrane by means of a water-tight chamber, *LS*, which surrounds the ma-

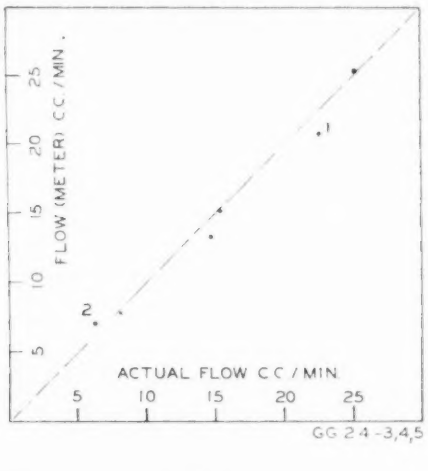
nometer tip. This is constructed of Lucite to aid in removing air bubbles. A plane mirror, M, (made from a small piece of a Bureau of Standards certified counting chamber coverslip) is coated on its silvered surface and sides with a special adhesive in order to prevent separation of the silver when the mirror is immersed. To make hysteresis minimal the mirror is mounted on the membrane by means of a tiny hard rubber peg and adhesive. Light enters the chamber and is reflected back through a +0.5D planoconvex lens, L, made at least 1.5 mm. thick to prevent warping. Since it is frequently impossible to mount the mirror parallel with the lens, the Locke's solution, with which the chamber is filled, forms a prism which separates the color components of the projected light. To correct this a small angled prism, P, capable of rotation through 360° is mounted in front of the lens. The differential manometer is mounted in the carriage of a Gregg manometer (9, 10). The side tubes, I, serve for filling and flushing the manometer. We have not found it necessary to separate the chamber from the blood by an extra rubber membrane (see 7, 8). The aortic pressure is recorded simultaneously with the flow by means of a Gregg pressure manometer (9, 10). As in the case of the flow meter a combination of a flat mirror mounted on the rubber membrane and a planoconvex lens of proper dioptre reflects the light beam to the camera.

Critique of apparatus. Because of eddy currents induced by the orifice plate, 50 per cent or more of the differential pressure head is permanently lost. To minimize this loss the membrane of the differential manometer is made very sensitive so that a large orifice and therefore small differential pressure can be used. To test the actual loss of pressure in an experiment two pressure manometers (M_1 and M_2, fig. 1) are usually connected to the blood stream some distance above and below the meter. In actual practice the pressure loss may reach 1–6 mm. Hg for a flow of 60 to 80 cc. per minute. In figure 2B, obtained from a mechanical setup in which a pulsatile flow was directed through the meter, the loss of head was 5 mm. Hg for a flow of 50 cc. per minute.

When carefully filled so as to avoid air bubbles the assembled meter has a natural frequency between 70 and 120 dv. per second (tested by elevating slightly the pressure on one side of the membrane and then suddenly allowing the pressures on the two sides to become equalized through the orifice connections). Figure 2C is a reproduction of a frequency curve obtained by this method when the sensitivity was 83 mm. deflection for a 10 mm. change of pressure with the camera at 4 meters.

Calibration. During every set of records a short segment of zero flow is recorded to detect any shift of the relationship between the flow and base line beams. At periodic intervals a complete calibration is made by disconnecting the orifice from the coronary circuit and driving Locke's solution or blood through it at measured rates of flow while recording the deflection of the flow beam.

The accuracy of such calibrations has been tested in two ways (see fig. 3). 1. The deflection of the beam from the zero position is plotted on log paper against the rate of flow at each of a series of flows. In most



A

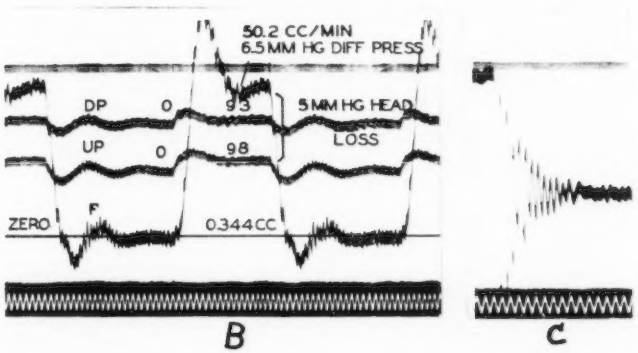


Fig. 2. A, comparison of actual flows with those calculated from flow records. B, example of the type of flow curve used in preparing A. The irregular contour was purposely obtained by adjusting the action of the mechanically operated stopcock which interrupted the inflow to the meter. C, typical frequency curve of the assembled differential manometer and orifice.

instances the points lie along a straight line with a slope of 2:1 indicating that the deflection varies as the square of the flow. 2. Using a high perfusion pressure the rate of flow is controlled by a valve placed first on the upstream and then on the downstream side of the meter, i.e., with first

a low and then a high potential head of pressure in the orifice. In each case the points plot along the same straight line. Changing either the sensitivity of the differential manometer membrane or the size of the orifice displaces the plot but does not alter its slope. When blood is used as the measuring fluid the points fall along the same straight line as those yielded by Locke's solution.

Interpretation of records. The rate of flow at any instant in a recorded curve can be determined by measuring the deflection of the flow beam

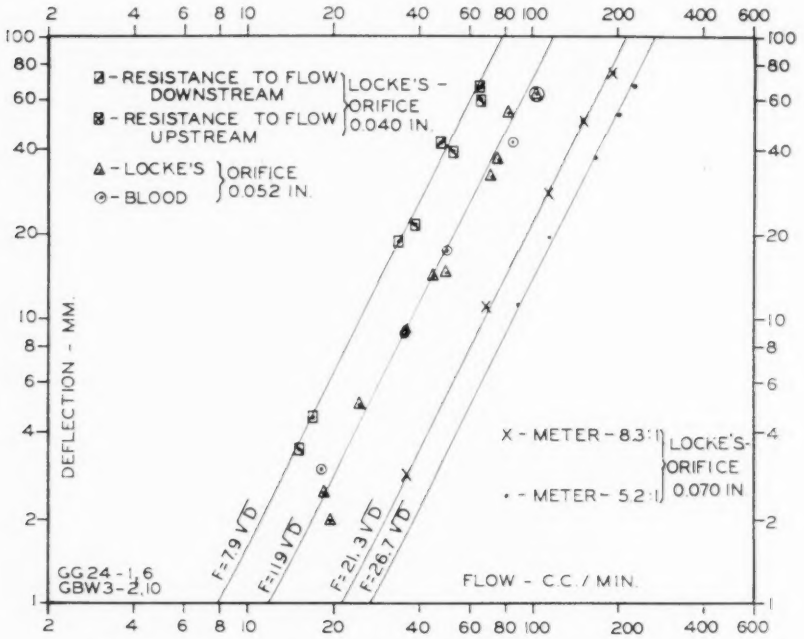


Fig. 3. Tracing of a plot of several flow calibrations on log log paper. Rate of flow (abscissa) in cubic centimeters per minute; deflection of flow beam from the zero position (ordinate) in millimeters. See plot for legend. Discussion in text.

from the zero position and reading off the flow from the plot of the calibration. To determine the total flow during an interval of time it is necessary to determine the average rate of flow for that interval. This may be done conveniently by dividing the area bounded by the flow curve, the zero position and the desired time ordinates by the horizontal distance in millimeters. However, since the deflection of the recorded flow curve varies as the square of the rate of flow, the curve first must be corrected to a linear ordinate scale. This may be done by replotting the ordinate

values of successive points on the recorded flow curve against a linear scale. The curves may be corrected more expeditiously by the use of a square root extractor such as that devised by Broemser (11) and Green (12).

To test the accuracy of the calibration for pulsatile flow the meter was perfused by a stream of fluid under constant pressure which was periodically interrupted by a mechanically operated stopcock. The frequency and speed of interruption, and the rate of flow were varied over wide limits in order to give a variety of contours to the resulting flow curve. Records (see fig. 2B for specimen) were made of the deflections of the flow beam together with collection in a graduate of the fluid flowing through the meter. The total flow for the experimental period was calculated from the recorded flow curve and compared with that which was directly measured. Figure 2A is a plot of several such comparisons. The agreement is good.

Technical procedures. Successful experiments were performed on 17 dogs. The animals were anesthetized with morphine and sodium barbital or sodium pentobarbital. Artificial respiration was begun, the heart was exposed, the main left descending coronary ramus and an adjacent side branch suitable for cannulation were dissected free. After rendering the animal's blood noncoagulable by the intravenous injection of heparin 75 units per kgm. plus chlorazol fast pink 80 mgm. per kgm., the subclavian artery and the side branch of the left coronary artery were cannulated and connected to the meter and manometers. An electrically operated clamp was placed around the central coronary vessel as shown in figure 1. Closing stopcocks (not shown in fig. 1) between the manometers (*M1* and *M2*) and the meter caused the manometers to record the aortic and peripheral coronary pressures respectively and thus gave us the differential pressure records previously described (1, 2, 3) for comparison with the flow records.

Results. *A normal flow curve.* Figure 4, segment 1, reproduces in the lowest curve a normal flow record obtained from the ramus descendens anterior, in the middle curve the downstream pressure, and in the upper curve the upstream pressure. Slightly sloping lines indicate simultaneous points in the curves. At the end of the first diastole, *A*, blood is flowing into the coronary artery at a rate of 28 cc. per minute. Approximately at the onset of isometric contraction, *A*, the rate of flow abruptly begins to diminish, and soon the flow line passes below the zero line, the maximum backflow being at the rate of 7.5 cc. per minute. With the onset of ejection from the ventricle and the rise of aortic pressure, *B*, the backflow diminishes and is rapidly converted to forward flow which reaches a maximum (40 cc. per minute) shortly before the peak of the aortic pressure curve. It then again declines leveling off during the latter part of systole at approximately 20 cc. per minute. Coincident with the closure of the

aortic valve and onset of isometric relaxation, *D*, the inflow again rapidly augments (within 0.04 sec.) to 40 cc. per minute and thereafter gradually declines with the diastolic fall of aortic blood pressure. As mentioned earlier, the difference of lateral pressure above and below the meter even at the moment of most rapid flow (i.e., early in diastole) is quite small (2 mm. Hg). During the period of backflow the downstream manometer, as expected, records a pressure higher than the upstream manometer.

Comparison of the flow curve with the differential pressure curve. In segment 3 of figure 4 is recorded the contour of the peripheral coronary pressure curve, together with the diastolic value (21 mm. Hg), while in segment 2 the systolic value is determined as 89 mm. Hg.[3] Figure 5A demonstrates the construction of the corrected peripheral coronary pressure

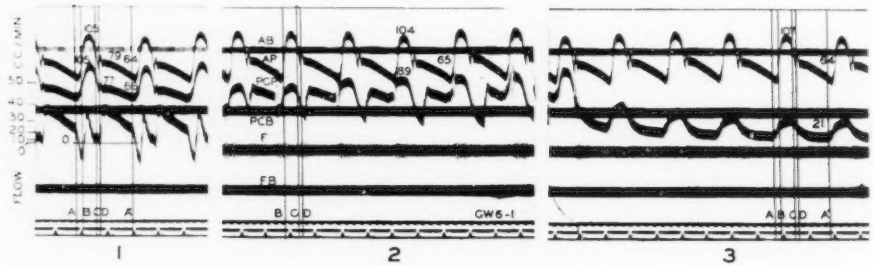


Fig. 4. 1, a typical flow curve. 2, determination of the systolic peripheral coronary pressure. 3, determination of the contour and diastolic value of the peripheral coronary pressure curve. *AB*, aortic pressure base line; *AP*, aortic pressure curve; *PCP*, peripheral coronary pressure curve; *PCB*, peripheral coronary pressure base line; *F*, flow curve; *FB*, flow base line. Figures along aortic and coronary curves pressure values for systole and diastole in millimeters of mercury. Calibration at left—rate of flow in cubic centimeters per minute—read to top of line. For other details see text.

curve, *PCP*, from the recorded peripheral coronary pressure curve (dotted line, traced from next to last cycle of figure 4, segment 3). *DP* is the differential pressure curve obtained by arithmetic subtraction of curve *PCP* from the aortic pressure curve *AP*.

Segment B, figure 5 (dotted line), is a trace of the recorded flow curve in figure 4A. *F* (solid line) is the same curve corrected to a linear ordinate scale. For comparison this curve and the differential pressure curve of segment A have been put together in segment C, figure 5, by so adjusting their ordinate scales that they coincide at the zero points and at the rate of flow recorded at the end of diastole. The shaded areas represent the differences between the two curves. From the standpoint of the

[3] See the previous papers (8) for discussion of the method.

recorded flow curve the important differences are: 1, a more marked deceleration and the occurrence of actual backflow during isometric contraction

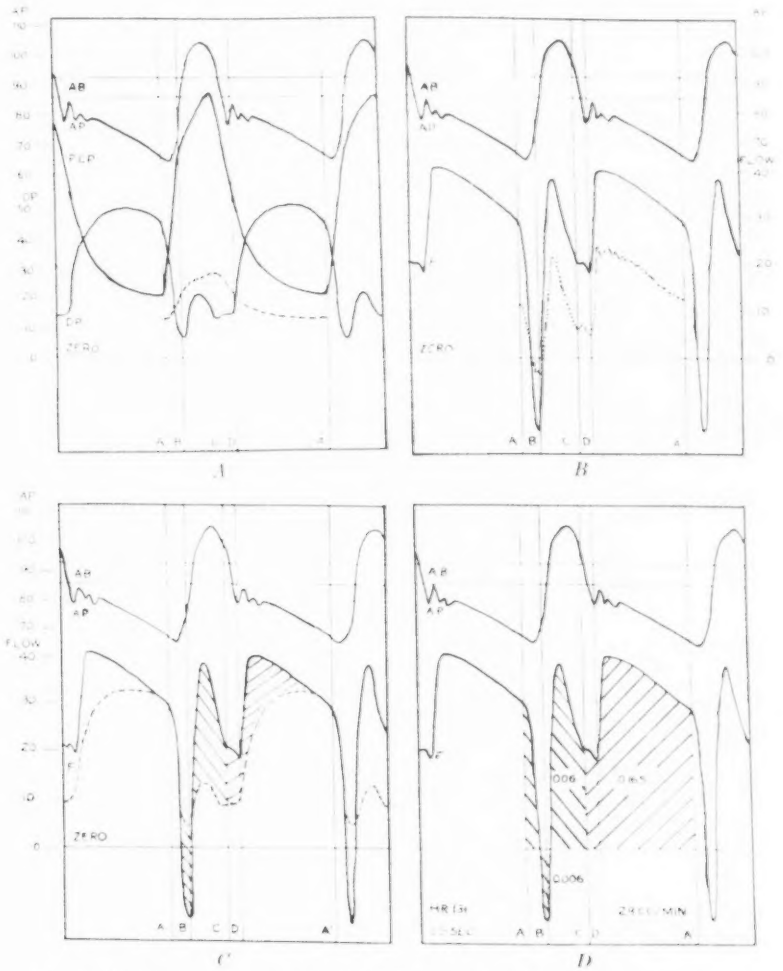


Fig. 5. Diagrams: *A*, reconstruction of a peripheral coronary pressure curve. *B*, tracing of flow curve, dotted line; reconstructed so as to have linear ordinate values, solid line, *F* (compare scale at left with that in fig. 4). *C*, comparison of differential pressure curve with flow meter curve. *D*, method of measuring area under the flow curve to get total flow. See text for details.

(A B), and 2, a greater forward flow during the rest of systole, during isometric relaxation and during early diastole.

The distribution of the total flow. Measurement of the area under the recorded flow curve (segment D, fig. 5) gives a total forward flow during systole of 0.06 cc. and a backflow of 0.006 cc. or a net flow of 0.054 cc. The total flow during diastole is 0.165 cc. or about three times that during systole. With a heart rate of 131 the total cyclic flow is 29 cc. per minute. In a series of experiments on different animals the "normal" systole: diastole ratio of flow varied from 1:1.75 to 1:7.4 with an average figure of approximately 1:2.4. Total flows ranged from 5.7 to 35 cc. per minute with an average figure of approximately 21 cc. per minute.

Discussion. While the flow curve in figure 4 represents the normal inflow into a coronary artery, the use of the moment to moment rate of inflow to interpret the mechanisms affecting flow requires detailed analysis. During a considerable portion of the cardiac cycle the rate of inflow and of intramural flow must differ. The main factors controlling intramural coronary flow are probably: 1, the aortic head of pressure; 2, the resistance to flow, which in turn is dependent upon the degree of contraction of the intrinsic muscles of the coronary vessels and the extent of extravascular compression or support. The factors causing the total rate of inflow to be greater or less than the intramural flow are: 1, the compressor action of ventricular systole on coronary vessels, and 2, the volume elastic effects produced by the pulsating aortic pressure. With these five factors in mind it will be our purpose to see how far the flow curves presented here can be interpreted.

The picture we conceive is as follows: During isometric contraction and early ejection the blood in the deeper lying and more strongly compressed coronary vessels is forced backward into the larger proximal channels and by thus contributing to the supply of blood available for the less strongly compressed and more superficial vessels reduces the inflow from the aorta. This fact is demonstrated by the rise of coronary peripheral pressure and by the backflow recorded by the constant pressure flow meter at appropriate perfusion pressures during isometric contraction and early ejection (5). As the aortic pressure rises, the extramural flow increases due to the increased distention and therefore greater capacity of the more superficial vessels. This effect also ceases at the peak of aortic pressure or slightly later, due to the inertia of the moving column of blood.

During isometric relaxation and early diastole the compressed myocardial vessels are rapidly released, thus causing the total inflow to exceed the actual intramural flow. As the aortic pressure drops, the expansion of the superficial coronary vessels is slowly reduced, thus decreasing the total rate of inflow below the intramural flow. At the end of diastole both effects approach zero.

The magnitude of these effects is large enough to produce serious

alterations of the inflow curve. Constant flow meter studies (5, 6) have shown that ventricular contraction and relaxation may reduce the inflow during systole by 50 per cent and augment early diastolic flow by 25 to 50 per cent. Volume elastic studies made on the coronary vessels (3) indicate similarly that the change in capacity of the vessels as a result of the cyclic change of aortic pressure may reach 25 or 50 per cent of the systolic flow.

From such an analysis it is deduced that during the last part of systole and diastole respectively the factors relating to extramural flow are largely removed and hence the metered inflow may approximate a true measure of intramural flow. In the actual records of flow (figs. 4, 5) the points probably representing intramural flow would be C or D for systole and A for diastole.

In addition, during these two periods separate estimates can possibly be made of the two important factors controlling intramural flow, i.e.,

TABLE 1

NUMBER	AORTIC PRESSURE		DIFFERENTIAL PRESSURE		*INTRAMURAL FLOW, CC. PER MINUTE		INTRAMURAL FLOW / DIFFERENTIAL PRESSURE		AORTIC PRESSURE / INTRAMURAL FLOW		CONDITION
	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	
1	119	86	12	71	9	22	0.75	0.31	12.1	3.9	Normal
2	140	100	22	85	15	31	0.68	0.37	9.3	3.2	Normal
3	96	70	24	56	26	37	1.10	0.66	3.7	1.9	Normal
4	135	75	43	56	32	37	0.74	0.66	4.2	2.0	Normal

* Flows and differential pressures determined for systole at (C) and for diastole at (A).

the state of contraction of the coronary vessels and their extravascular support. This is possible because at the end of diastole, extravascular compression is at a minimum, while at the height of intraventricular pressure, compression is at a maximum. Comparison of the intramural flow at the latter time with that late in diastole gives a qualitative estimate of systolic extravascular contribution to the control of coronary flow.

Since the intramural flow was found proportional to the differential pressure (5) it is to be expected that with the reservations outlined above the flow curve given by the orifice plate meter should be patterned after the differential pressure curve. Figure 5, segment C, shows that the agreement is good, especially as to direction of movement and time relation of the changes. The differences in amplitude emphasized by the shaded areas approximate in magnitude and agree in direction with those predicted in the above discussion. However, one very definite difference appears: the ratio of flow to differential pressure at the end of systole, i.e.,

at that portion of the cycle when it presumably indicates intramural flow, is greater than the same ratio at the corresponding interval at the end of diastole (compare the amplitudes of curves *F* and *DP* at the points indicated by the simultaneous ordinates C and A or A^1 in segment C of figure 5). As evident from representative data in table 1 this is the usual occurrence in different experiments. The true reason for this difference has not yet been ascertained.

Table 1 also shows the ratio of the aortic pressure to the simultaneously recorded rate of flow in cubic centimeters per minute for the same points in the cycle. Comparison of these two shows that the onset of systole increases the peripheral resistance by 2 to 4 times that present in diastole.

SUMMARY

1. A method is described for continuous optical registration of the instantaneous rate of inflow into a coronary artery. This involves shunting the blood from the aorta to the coronary artery through a short external circuit containing an orifice (or other device) connected with a differential manometer.

2. The left coronary inflow curves so obtained demonstrate that beginning approximately at the onset of isometric contraction there is a rapid retardation of flow but that with the rise of aortic pressure during ejection the inflow rapidly accelerates, reaching a peak during the middle of the rise of aortic pressure and then declining to a more or less constant rate of inflow during the latter part of systole. Following the incisura there is again a rapid acceleration, the inflow reaching a peak early in diastole and then declining with the progressive fall of aortic pressure in diastole.

3. The inflow records are complicated by volume elastic effects due to the cyclic rise and fall of aortic pressure and by a compressor action of ventricular systole.

4. Despite these complications, and unless some other unknown factors are operating, it seems probable that the rate of inflow at the end of diastole just preceding isometric contraction, can be used as an index of intramural flow during diastole.

5. Similarly it seems probable that the rate of inflow during the brief interval at or just preceding the onset of protodiastole, i.e., at the peak of the peripheral coronary pressure curve, can be used as an index of the systolic rate of intramural flow. In almost all instances the systolic intramural flow so measured is of sizable magnitude.

6. The rate of intramural flow per millimeter of differential pressure is greater during systole than during diastole.

7. Simultaneous measurement of aortic pressure and rate of intramural flow indicates that the resistance to blood flow existing during the latter part of diastole is increased from 2- to 4-fold during systole.

8. The total flow may be determined from the moment to moment flow curve by appropriate procedures.

The authors wish to express their appreciation to Dr. R. Wegria for assistance in the performance of several of these experiments.

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CHANGES IN THE CORONARY CIRCULATION FOLLOWING INCREASED AORTIC PRESSURE, AUGMENTED CARDIAC OUTPUT, ISCHEMIA AND VALVE LESIONS[1]

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In previous work (1, 2) the manner in which the relative systolic and diastolic flows through the left coronary are modified under various dynamic conditions, normal and abnormal, were estimated by analysis of differential pressure curves taken from a coronary ramus. Since we have now found with a constant pressure meter (3, 4) that such pressure differentials can alter less than the flow, we have repeated these experiments registering simultaneously pressure differentials and inflow by means of the orifice meter described in the preceding communication (5).

The results of typical experiments are grouped together in figure 1 and the essential data taken from each of these curves are given in table 1 under corresponding letters.

Elevation of aortic pressure. Blood pressure was elevated by compression of the thoracic aorta. As shown by comparison of records in figure 1, A and B, the systolic and diastolic flows increase significantly. The minute flow, i.e., the sum of the systolic and diastolic flows times the heart rate, increases from 10.3 to 36.4 cc. per minute during the increase in mean pressure from 22 to 91 mm. Hg. This confirms previous work on differential pressures (1). However, the increases are proportionately much less than the elevation of aortic blood pressure. If the ordinate lines A and B are regarded as indicating the times when coronary flow is largely intramural (5) both systolic and diastolic intramural flows are augmented, the former from 0 to 24 cc. per minute and the latter from 14 to 43 cc. per minute. However, as shown in table 1, A, B, the systolic flow increases more than and the diastolic less than the corresponding differential pressures.

Increased cardiac output. (C and D of fig. 1 and table 1.) When blood warmed to body temperature is slowly infused into the jugular vein the

[1] Preliminary reports of these experiments were presented before the American Physiological Society at the Toronto meeting April 27, 1939, and before the Cleveland Section of the Society for Experimental Biology and Medicine, March, 1939.

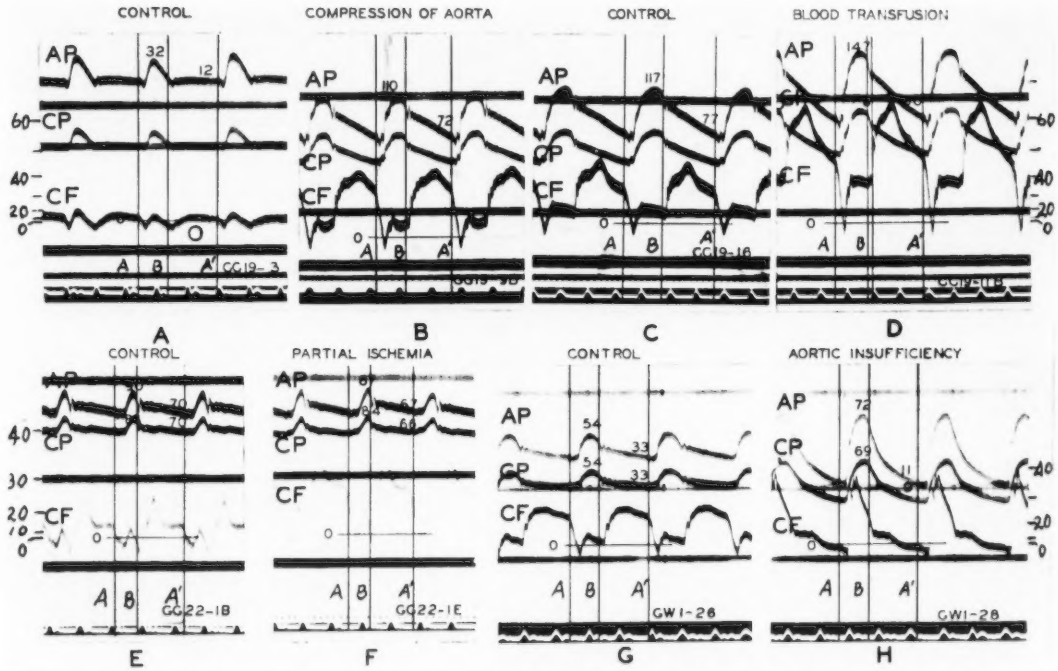

Fig. 1. Records showing the effects on coronary blood flow of aortic compression (B), blood transfusion (D), ischemia of the vascular bed (F), and aortic insufficiency (H). Respective controls are *A*, *C*, *E*, *G*. Vertical lines (*A*, *B*) indicate approximate ends of diastole and systole. *AP*, aortic pressure, *CP*, coronary pressure, *CF*, coronary flow. Time $\frac{1}{5}$ second. Calibration accompanying each pair of records = rate of flow in cc. min.

minute coronary flow increases from 30 to 49 cc. per minute. Only a slight increase of peripheral coronary pressure occurs despite the considerable elevation of aortic pressure and as a result the differential pressure increases considerably both in systole and diastole. The increase in systolic flow recorded by the meter is approximately proportional to the increase of differential pressure but in diastole the flow increases more than the differential pressure.

Ischemia of the myocardium. In the experiment illustrated in figure 1 and table 1, E and F, a coronary ramus was occluded for about 30 seconds. Upon restoration of the blood flow the total and intramural flows showed large increases both during systole and diastole, while the aortic pressure and peripheral coronary systolic and diastolic pressures decreased con-

TABLE 1

RECORD NUMBER	HEART RATE	AORTIC PRESSURE		AORTIC PRESSURE AT 1/10	PERIPHERAL CORONARY PRESSURE		DIFFERENTIAL PRESSURE		INTRAMURAL FLOW, CC. PER MINUTE		TOTAL FLOW, CC.		INTRAMURAL FLOW DIFFERENTIAL PRESSURE		CONDITION
		Systole	Diastole		Systole	Diastole	Systole	Diastole	Systole	Diastole	Systole	Diastole	Systole	Diastole	
		C.			C.		C.		C.		C.		C.		
A	114	32	12	18	25	3	−7	9	0	14	0.0235	0.067	0	1.56	Control
B	115	110	72	108	85	15	23	62	24	43	0.077	0.24	1.04	0.69	Aortic compression
C	90	117	77	115	85	16	30	62	23	36	0.115	0.245	0.77	0.58	Control
D	89	147	90	140	90	16	50	74	40	50	0.16	0.395	0.80	0.68	Blood transfusion
E	131	90	70	80	75	22	5	48	0	15	0.017	0.125	0	0.21	Control
F	139	87	67	80	69	11	11	47	14	27	0.052	0.17	1.28	0.575	Ischemia
G	119	54	33	50	50	10	0	23	12	25	0.047	0.14	∞	1.09	Control
H	110	72	11	70	55	10	15	−1	26	0	0.089	~~0.56~~ 0.056	1.73	0	Aortic insufficiency

siderably. The systolic differential pressure rises but the diastolic remains unchanged. As a result, the flows increased much more than the differential pressures. The minute flow increases from 17.3 to 30.8 cc. per minute.

Aortic insufficiency. We have confirmed a previous observation (2) that in aortic insufficiency the differential pressure increases during systole and decreases during diastole. The metered flows vary in the same direction but change much less proportionately than the pressure differential (G and H of fig. 1 and table 1). In the example shown here due to the extreme fall of diastolic pressure the increase of systolic flow does not compensate adequately for the lower diastolic flow, and hence the minute flow decreases from 22.5 cc. to 16 cc. per minute.

Aortic stenosis. Several observations made before and during this

disturbance indicate that the flow changes correspond closely with those predicted from the differential pressure curves, namely, that there is little effect on diastolic flow but a marked reduction of systolic flow.

SUMMARY AND CONCLUSION

Records of the moment to moment rates of flow and of the total inflow into the left coronary artery of dogs have been taken with the orifice meter together with the aortic and peripheral coronary pressures under different dynamic conditions.

Study of such indicates that during both systole and diastole the total and intramural flows increase following aortic compression, blood transfusion, ischemia and aortic insufficiency (only during systole), while in diastole of the latter the intramural flow decreases and the total flow increases. The pressure differentials follow in the direction of the metered flows but since they change much less they can provide only a qualitative measure of flow. These differential pressure changes may be less than, greater than or the same as the flow alterations.

The latter findings permit certain deductions, provided one subscribes to the idea previously advanced (5) that changes especially during diastole, in the ratio of intramural flow to differential flow may indicate alterations in size of the available coronary bed, and in addition that changes in systolic peripheral coronary pressure reflect changes in extravascular compression or support.

Calculations made upon this basis indicate that following increase of cardiac work through simple elevation of aortic pressure the available coronary bed becomes smaller while in ischemia and in augmented cardiac work due to increased cardiac output the bed increases, because in the former the diastolic flow increases less than the pressure differential while in the latter the reverse is true. Substantiating this is the observation that the minute flow per millimeter Hg aortic pressure decreases with aortic compression and increases with augmented venous return.

In elevation of aortic pressure, augmented cardiac output, aortic stenosis and aortic insufficiency, but not ischemia, the extravascular support is presumably increased as evidenced by the increased peripheral coronary systolic pressure. However, failure of such increase in extravascular compression to rise concomitantly with the aortic systolic pressure is in part responsible for the augmentation of systolic flow in these conditions and its converse for the reduction of flow in aortic stenosis.

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OBSERVATIONS ON THE GENESIS OF THE ELECTRICAL CURRENTS ESTABLISHED BY INJURY TO THE HEART[1]

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While considerable work has been done on the electrical potentials established by injury to the heart (reviewed by Schütz (1)) and an analysis of the changes so produced in the electrocardiogram has proved fruitful in clinical practice (cf. Bohning et al. (2), for example), certain aspects of the problem are still unsettled. This is particularly true of the genesis of the characteristic T wave deformity that occurs after injury.

In the present report, our observations from two types of experiments will be discussed. In the first the course of electrical events was followed in a spot on the mammalian ventricle during the development of and recovery from an injury produced by compression. In the second, the course of electrical events was followed in a series of approximately equidistant spots within and outside of an injury produced by injection of 95 per cent alcohol.

I. *The course of electrical changes during the production of and recovery from a small injury in the dog's heart.* Two experiments were done with identical results. The animals were anesthetized with nembutal (25 mgm./kilo), the anterior chest wall removed and artificial respiration maintained. The pericardial sac was opened and arranged to cradle the heart. Unipolar leads were used with the indifferent electrode placed beneath the skin of the left leg and the direct electrode on the surface of the right ventricle. Both electrodes were non-polarizable, and each consisted of a wick soaked in saline-agar and fastened to a porcelain boot filled with zinc sulfate solution into which a zinc electrode was placed; the copper lead wire was attached to the zinc electrode. The connection to the electrocardiograph was such that negativity of the cardiac electrode gave an upward deflection in the record. The wick of this electrode was incorporated in a pressure electrode, consisting of a small glass tube, tapered at the end in contact with the heart (previously described, Jochim, Katz and Mayne, 3); the wick was passed through the tube and projected about 0.5 mm. from the tip so as to pick up the electrical currents from

[1] Aided by A. D. Nast Fund for Cardiac Research.

the center of the injury. The records were standardized so that 10 millivolts gave a deflection of ½ cm.

After placing the electrodes, the one on the heart very gently so as not to cause injury, a continuous record was made. During the course of the recording, gradually increasing pressure of a degree sufficient to cause injury was manually applied on the heart electrode (cf. Jochim, Katz and Mayne, 3), and then later, the pressure was gradually released. This maneuver was repeated several times.

In figure 1 a typical experiment is shown with segments of the record before injury (A), during increasing injury (B), during the injured state (C), during recovery from injury (D), and after recovery had been completed (E). The sequential changes in potential of every second beat in

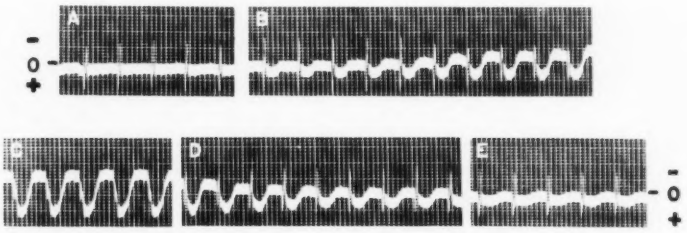

Fig. 1. Segments from a continuous record of a unipolar lead from a spot on the dog's ventricle showing changes occurring during the development of and recovery from a small injury produced by pressure on the special cardiac electrode. A, control before pressure was applied; B, during application of increasing pressure; C, during period of maximum injury; D, during release of pressure; E, after release of pressure, showing almost complete recovery.

Indifferent electrode was on left hind leg. The intervals between successive segments of the record ranged from approximately 2 to 10 seconds. The isoelectric level for the top of the line is marked 0; upward deflection = negativity (−) of cardiac electrode, downward, positivity (+). Discussed in text.

one experiment where compression was applied and released twice are charted in figure 2. The upper row of dots represents the negative potential of the injured area during diastole of the ventricles at the moment just before the beginning of the QRS complex. The negativity was measured with respect to the potential of this spot on the heart at the same moment before injury, i.e., the potential of the resting uninjured muscle. The lower row of dots represents the maximum positive potential developed at the injured area during ventricular systole, i.e., at the lowest level of the S-T depression; the same reference potential was employed as that used for the upper row of dots. In these experiments it is assumed that the potential of the "indifferent" electrode remains essentially constant (Wilson et al. (4, 5)). Since each millimeter of the record

equalled 2 millivolts the potentials would be twice that of the ordinate scale. Since the cycle length was 0.25 sec., each dot represents 0.50 sec. in time.

Inspection of these two figures will show that this compression type of injury produced the following almost entirely reversible changes in the potentials of the injured area:

1. A decrease in QRS amplitude involving the upright phase more than the inverted one; the persistence of this part of the electrical curve

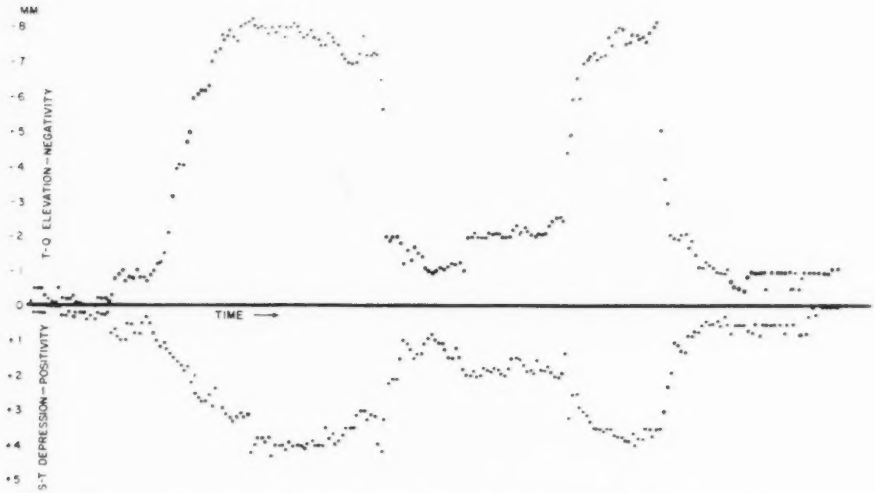

Fig. 2. Chart showing the time course of the development and regression of the T-Q elevation and S-T depression caused by the development of and recovery from injury produced with the pressure electrode. Two periods of compression and decompression are shown. The maximum displacement in millimeters of the T-Q and S-T intervals of every second beat from a continuous record were plotted against time on the abscissa. Since the cycle length was 0.25 sec., the time interval between any two successive points is 0.50 sec. The zero line represents the potential of normal resting muscle, i.e., just before the beginning of the QRS in the control record before compression. Discussed in text.

was responsible for the notching of the downstroke of the full blown monophasic deflection (seg. C., fig. 1).

2. The development of a depression (positivity) of the S-T segment (with the disappearance of the T wave) below the isoelectric line of the curve before injury. Occasionally, a slight residue of this change persisted after the injurious compression on the electrode was relieved (seg. E., fig. 1).

3. The development of an elevation (negativity) of the T-Q interval above the isoelectric line of the control curve before injury.

1. The extent of the S-T and T-Q level shifts were not equal but they followed a similar time pattern of development and disappearance.

Our observations confirm those recently made by Eyster, Meek, Goldberg and Gilson (6) using different methods of producing injury and lend support to their view that injury causes the development of a potential distribution which can be explained on the assumption of the existence of two concentric rings of charges, the inner one negative and the outer one positive during ventricular diastole, and the reverse polarity during ventricular systole. We have explored the field in model experiments

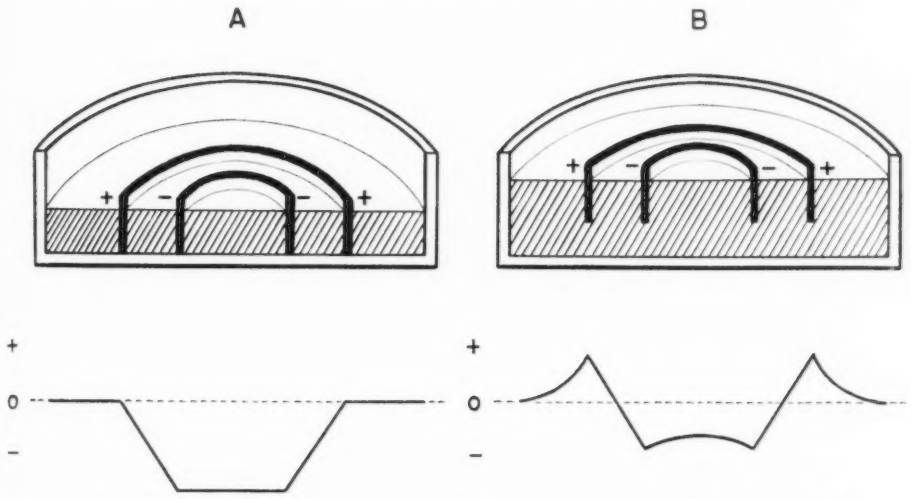

Fig. 3. Diagram showing the potential distribution in a saline field produced by two concentric rings of opposite charge. In A, the rings are fastened to the bottom of the dish so that the saline is divided into 3 separate compartments. This arrangement gives the potential distribution, shown below diagram, calculated by Eyster, Meek et al. (6). In B, the rings are merely immersed below the surface of the saline without breaking its continuity. The resulting potential distribution is shown below the diagram. In each, the zero level represents the potential of a point on the edge of the field. Discussed in text.

with such concentric rings and have confirmed the potential distribution postulated by Eyster, Meek et al (6). It is important to point out that in order to obtain this potential distribution the concentric rings must be so placed in the field that they divide it into three separate regions having no communication with each other except through the rings themselves (fig. 3 A). If the rings are immersed in a saline field without interrupting its continuity, the potential distribution is as shown in figure 3 B, with a positive phase at the outer ring which falls off to zero as the periphery of the field is approached.

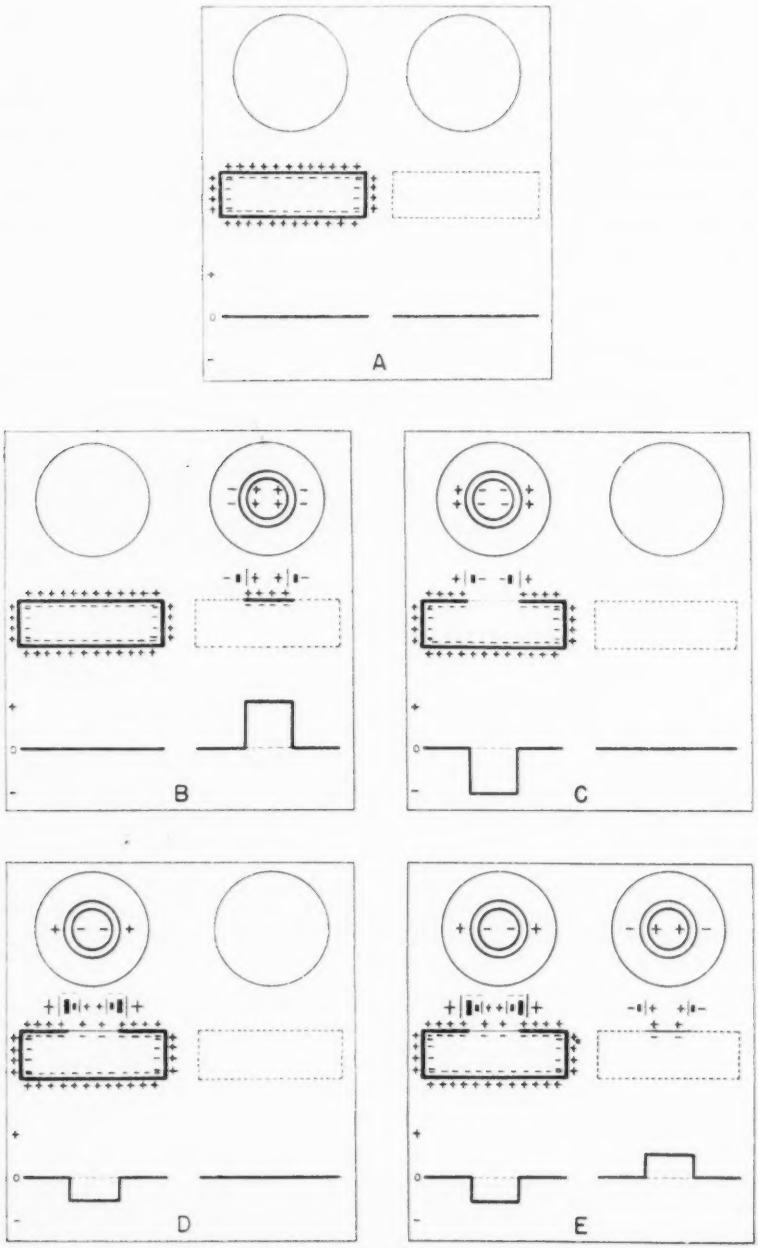

Fig. 4

This electrical solution of the effect of injury is readily related to the classical membrane theory. In figure 4, we have attempted to illustrate this correlation. In A the proposition is presented that at rest the electrical state of the syncytial membrane may be represented by a continuous polarized membrane with negative charges on the inside and positive on the outside; hence the external surface of the cell will be of zero potential during diastole, i.e., it will have the same potential as the indifferent electrode in the external field. During complete activation the polarized state being destroyed, no battery will exist and hence again the cell surface will be of zero potential, i.e., it will have the same potential as it had during rest.

However, several other possibilities exist: 1, a region of normal resting polarity may not respond during systole and remain polarized; 2, a region of complete depolarization may be caused by injury; 3, a region of partial depolarization may exist during diastole which responds during systole and has its depolarization completed, and 4, a region of partial depolarization may exist during diastole which does not respond during systole.

B of figure 4 shows the first of these possibilities. During diastole, this is like a normal cell but during systole the remaining polarized part of the cell will act as the source of current and give rise to a distribution of potential which can be produced by two concentric rings of charges, the outer ring negative and the inner positive. A monophasic action curve during the heart cycle could thus be obtained from this region without any injury current being present at rest. This has been described to occur by Ashman and Woody (7).

C of figure 4 shows the second of these possibilities. The injured area is completely depolarized at rest and hence a unipolar or bipolar lead from this region will show a current of injury, the polarized uninjured part of the cell acting as the current source. The distribution of charges can be represented by two concentric rings, the outer positive, the inner negative. During systole, this double ring will disappear since the entire

Fig. 4. Diagrams representing the distribution of *charges* on the two surfaces of a cell membrane at rest (on the left in each diagram), and during complete activation (on the right). The heavy solid lines of the cell wall represent complete polarization, the thin solid portions represent partial polarization, and the dotted lines indicate complete depolarization. Just above each cell is indicated the resultant battery produced by the assumed distribution of charges. Below each cell is shown the *potential* distribution across the upper surface of the cell, referred to a distant point in the field as zero. Above each cell is shown a circular field viewed from above with appropriate concentric rings of opposite charge to give the potential distribution shown. These rings are assumed to be placed in the field as in figure 3 A (some slight modification would have to be made if the rings were as in 3 B). A, normal cell; B, cell with a region *not responding during activation;* C, cell with a *region of complete injury;* D, cell with a *region partially injured but responsive;* E, cell with a *region partially injured and irresponsive.* Discussed in text.

cell will be depolarized. This state will also give rise to a monophasic curve from this region during the heart cycle.

D of figure 4 represents the third possibility. Here the injured area is only partially depolarized at rest. This is really a variant of the second possibility, the only difference being that the resting current of injury and the monophasic action current will be smaller in magnitude since during diastole the injured area will also act as a current source which will neutralize in part the effect of the uninjured part of the cell. During systole, both sources of current will be eliminated. No reports of the actual occurrence of the foregoing possibilities (C and D) have appeared in the literature.

E of figure 4 represents the fourth possibility. During diastole a current of injury will flow for the same reasons as in cases C and D; but during systole the lack of response of the injured region will cause a reversal of the polarity of the concentric rings since the injured part of the cell will be the only part of the cell which will serve as the current source. This is the type of monophasic curve which Eyster, Meek et al. (6) and we, ourselves, have obtained.

It is apparent from the above description that these newer observations are not contradictory to the classical view. The observations of Eyster, Meek et al. have, however, served to define the classical membrane theory in more precise terms. It has led us to the idea that regions may be injured, irresponsive, or both and while in each case monophasic curves occur, the potential changes will differ in each case.[2]

The concept outlined above to account for the changes in the electrical-time curve is not contradictory to the observations reported previously from this laboratory (Jochim, Katz and Mayne, 3). It is apparent that the bipolar lead from an injured to an uninjured area on the heart is the algebraic sum of the electrical variations from each as has recently been shown experimentally by Eyster, Meek et al (6). The onset of the monophasic curve in the bipolar lead used by us was thus the same point as that used in uninjured curves in unipolar leads (cf. fig. 5) and would, therefore, depend on when the activation in the uninjured area began. The onset and end of the monophasic curve in unipolar leads would be the same for all spots of the heart since as shown above it would be determined by the time at which depolarization begins and repolarization ends in the ventricles. The end of repolarization in the uninjured area is shown in the unipolar lead by the end of the T wave. In bipolar leads, this T wave summating with the curve from the injured area will alter the con-

[2] The changes in the potential of the QRS in unipolar leads is dependent, in all likelihood, upon the presence of irresponsive areas beneath and adjacent to the electrode causing injury, and the degree of change in QRS is no doubt a rough measure of this irresponsiveness.

tour and time of termination of the monophasic curves. It is thus apparent that the points used by us to measure the duration of the monophasic curve would be an index of the duration of activity of the uninjured area

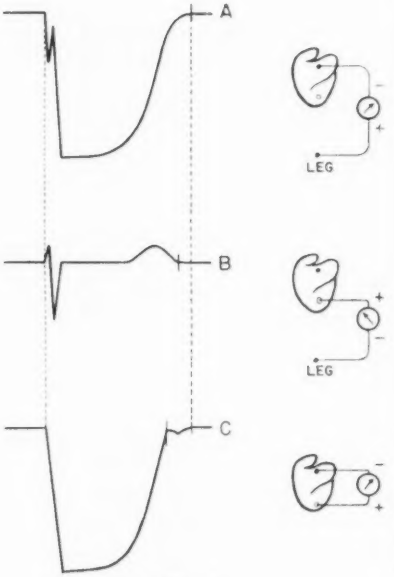

Fig. 5

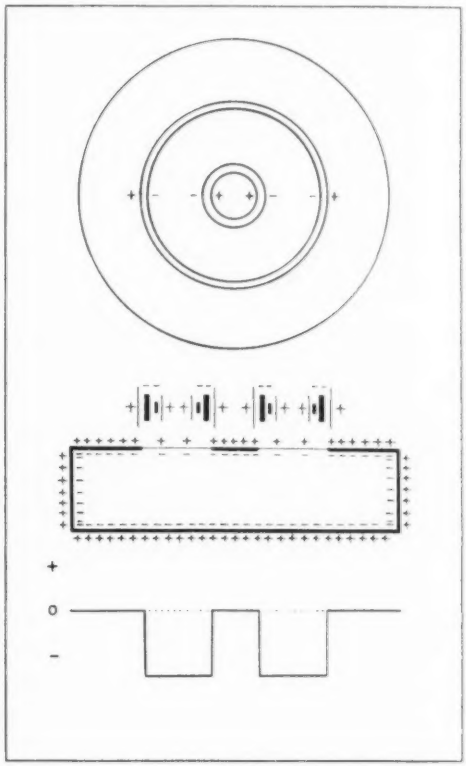

Fig. 6

Fig. 5. Diagram showing summation of a unipolar lead from an injured area (A) with that from an uninjured area (B) to give the resultant bipolar lead of different duration (C), diagram of connection to galvanometer is shown on the right of each curve. Discussed in text.

Fig. 6. Diagram with conventions as in figure 4 to illustrate the possible state of the cell membrane polarization during the inscription of the coronary type T wave found in the annular region around the injury resulting from the *temporary lag in repolarization of a partially injured responsive area.* Discussed in text.

and would vary with the location of the electrode on uninjured but not with that of the electrode on injured regions.

II. *The course of electrical events in a series of approximately equidistant spots within and outside of an injury produced by 95 per cent alcohol.* Ex-

periments were done on ten animals anesthetized with nembutal (25 mgm./kilo). The procedure of exposing and preparing the heart was the same as in the preceding experiments. Similar non-polarizable wick electrodes were used, those on the heart not being enclosed in the pressure electrode but, instead, permitted to just rest on the epicardium. These wicks were fastened to their respective spots by very fine epicardial sutures, care being taken to wait for complete recovery from this very mild injury before starting the experiment. Unipolar leads were used, and these were standardized so that 10 millivolts equalled $\frac{1}{2}$ cm. Normal control records were taken from a number of spots spaced equally along a straight line across the surface of the right ventricle. The records were taken in quick succession by connecting each electrode on the heart in turn through the galvanometer with the indifferent electrode by means of a rotary selector switch.

After the control records were taken, a localized area of injury was produced by the intramyocardial injection of 2 cc. of 95 per cent alcohol. This injured area usually extended beneath 2 to 4 of the spots on the heart upon which electrodes had been placed. The remaining spots extended over tissue near the margin of the injury and well out into normal tissue. Immediately after production of the injury, the records were again taken from exactly the same spots that were used before for controls. These were repeated at lengthening intervals until the records showed a return to the normal contour, a period of $1\frac{1}{2}$ to $4\frac{1}{2}$ hours.

The results of a typical experiment are shown in figure 7. In this experiment the analysis was integrated by making a series of 3 dimensional models (cf. fig. 8). Each model represents the time-space distribution of potentials before and at certain times after the production of the injury. For each model, one cycle of the record from a single spot was enlarged 5 fold, a cardboard backing pasted on each record and the curve cut out along its upper border. These cutouts were mounted vertically on a stand, the cycle from each spot 1 cm. behind the other, with the point just before QRS in each in the same horizontal and the same vertical planes. It is recognized, of course, that placing these points on the same horizontal level ignores the shift in potential of these spots as injury is induced, but this is not disturbing for the purposes of our analysis. The space between the cutouts was filled with modelling clay and smoothed over to make a three dimensional time-space-potential diagram. The axes represent: height, voltage; breadth, time in heart cycle; and depth, distance along heart surface. Before photography the models were coated with Alco-glaze and then painted white in order to preserve them and make reproduction easier. The models represent: A, control before injury; B, immediately following injury; C, D, E, F, G, and H, respectively 5, 15, 30, 45, 60 and 75 minutes after injury.

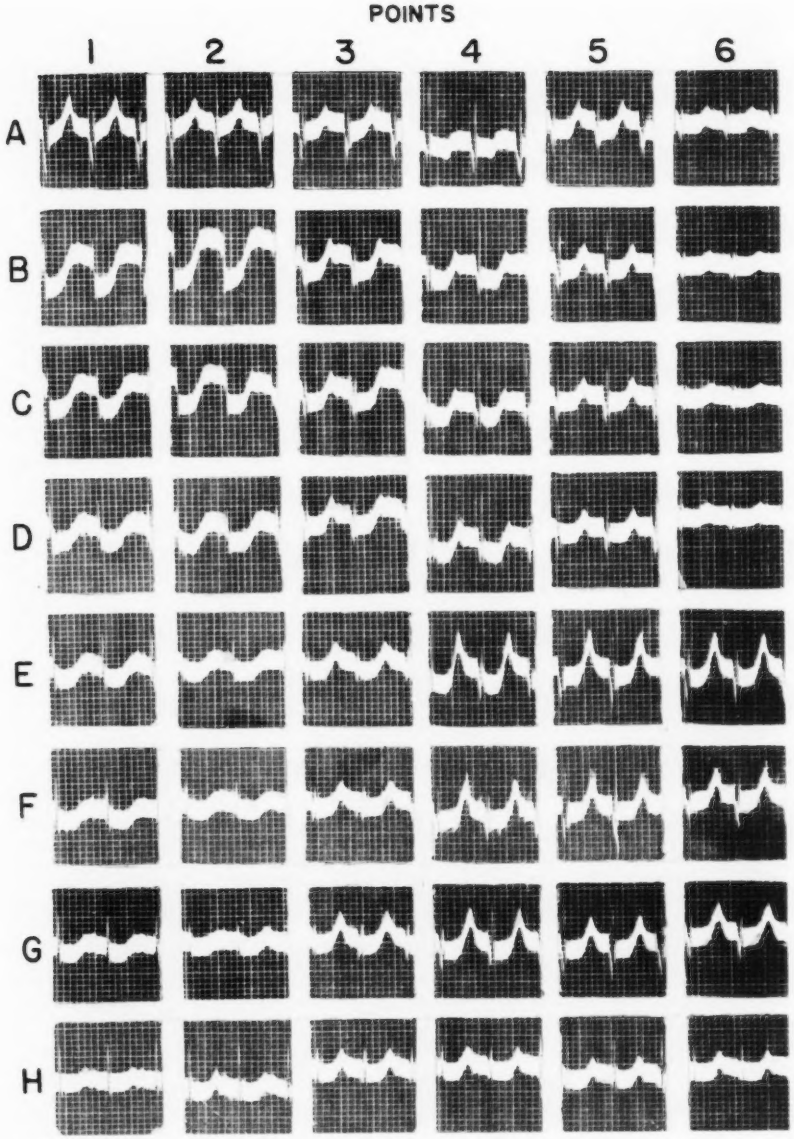

Fig. 7. Serial unipolar leads taken from 6 spots on the heart before and after intramyocardial injection of alcohol. Each vertical numbered column contains segments of records made from the same spot at various times. Spot 1 was approximately at the center of the alcohol-injected region; the remaining 5 spots were located in a straight line from spot 1 at approximately equal distances from each other spot, 5 being outside and 6 well out into normal tissue. Series A was taken before injection, and series B immediately afterwards. Series C, D, E, F, G, and H were taken 5, 15, 30, 45, 60, and 75 minutes after injection respectively. Discussed in text.

The results obtained can be summarized as follows:

1. Changes in the contour of the QRS occurred first in the region in which alcohol was injected and later also in the other areas outside this region. This indicates that the pattern of activation of the heart in this region is altered, first by the original injury and later, by the subsequent effect of the initial injury on neighboring regions. The outstanding change

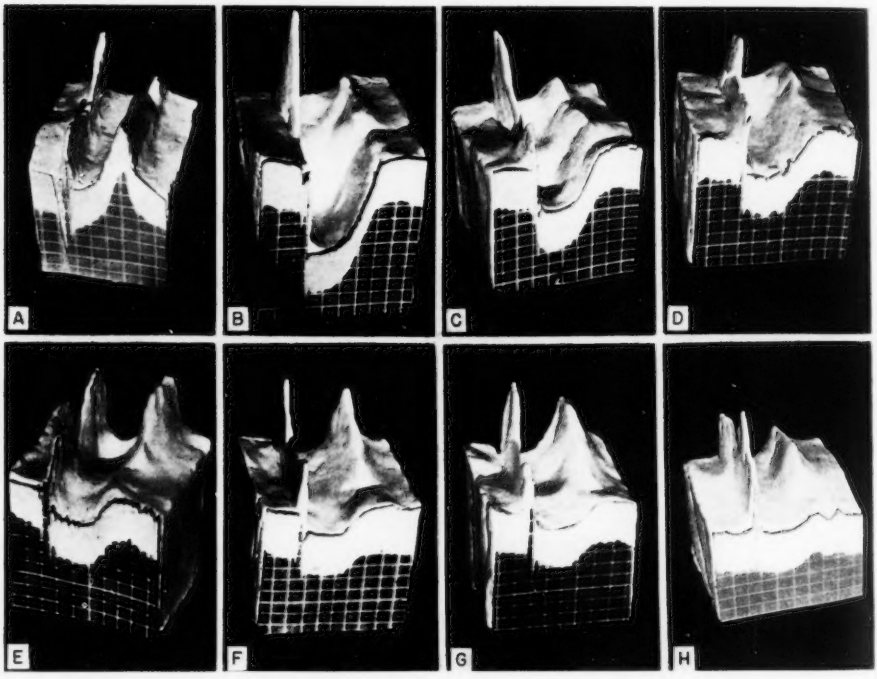

Fig. 8. Photographs of a series of three dimensional models, each model made from the correspondingly lettered series of figure 7. The horizontal axis represents time during the heart cycle; the vertical axis represents potential; and the axis at right angles to these two represents distance from the center of injury. Discussed in text.

was in the first inverted phase of the QRS and only slightly less was the effect on the subsequent positive phase. These experiments like those of Wilson et al. lend support to the view that alterations in the pattern of activation are probably responsible for the alterations in the QRS in indirect leads in animals and man which are seen to follow localized ventricular wall injury. In figure 8 these changes affect the contour of the precipitous QRS "canyon-mountain range."

2. Changes in the level of the S-T segment in the form of a depression at first confined to the region of the initial injury and its immediate environs and being greatest in the center and less marked at the edge. Later, as the S-T depression in the center of injury decreases, this difference between the center and the edges of the injury lessened and the area over which the S-T depression was located extended outwardly. Eventually, this S-T depression disappeared or tended to do so. The mechanism for its production would be along the lines mentioned in the possibilities discussed in section I and illustrated in figure 4. It is most likely that possibility D is what actually occurs. Obviously, as restitution occurs with time and a new polarized surface is gradually established in the injured region, the variations in potential of this region between the resting and active periods of the ventricles will decrease. Because the injury is less at first at the margin than in the center of the alcohol injected area the potential variation during these two periods will be less in the former region. The slower restitution at the margins of the original area and the later extension of the injured area is attributed to the spread by diffusion and via the lymphatics of noxious material from the area originally injured by alcohol. This chain of events gave rise in figure 8 at first to a deep broad "valley" which midway back sloped steeply upward; later, this was replaced by a shallower more extensive "valley" and ultimately it disappeared.

3. The most striking finding, hitherto not fully described as far as we know, was the late development of a characteristic upright T wave (seg. E., fig. 7) confined *to the margin and the region surrounding* the original injury. This T wave tended to wane later, more rapidly in the outermost regions and, at the same time, it tended to appear in regions closer to the center of original injury (seg. G, fig. 7). Ultimately, it too tended to disappear (seg. H., fig. 7). The contour of this T wave is specific for the "coronary" T wave described in man, namely, a peaked T wave, with symmetrical limbs, rounded shoulders and accompanied by an S-T segment which both deviates and bows in a direction opposite to the T wave. This T wave appeared in figure 8 as a broad "mountain range" in the back of the model which sloped downward from back to the front; later its tilt from front to back decreased, as did its maximum height and ultimately this T was replaced by the normal broken uneven T wave "range."

On a purely electrical basis, following the views for the S-T changes which Eyster, Meek et al. suggested and which are confirmed by our work, this T wave change must indicate the presence of two pairs of concentric rings at the time in the heart cycle when the T wave is recorded as shown in figure 6. The inner pair of rings would be located at the inner margin of the area where this T is recorded, with the positive charge on the inside and negative on the outside. The outer pair of rings would

be located at the outer margin of the area where this T is recorded and have the charges of each ring the reverse of the inner pair. This would limit this T configuration to the area enclosed between the inner and outer pair of rings.

This view can be correlated with the membrane theory. At the time the T wave is being written the process of restitution of the polarized state is occurring at the cell surfaces. It would, therefore, be logical to assume that the "coronary" T wave was an expression of an alteration in the pattern in which this restitution takes place, and it would readily be accounted for along the lines developed in section I and illustrated in figure 6 if it were assumed that the region where this T wave occurs was, because of injury, tardy in its restitution process while more or less normal in its responsiveness to activation. In other words, in the ring-like region surrounding the originally injured region, there is left a more or less normal depolarization process during activation, *but there is a retardation in the rate of repolarization so that this region temporarily lags behind the rest of the ventricles.* In figure 6 the membrane potentials are illustrated during this part of the heart cycle in a manner similar to figure 4.

It would appear from this hypothesis, that the "coronary" T wave in indirect leads following local myocardial injury in animals and in man could be explained as an electrical expression of the occurrence of regions in which the ability to respond to activation is not impaired to any extent but in which the injury has resulted in a retardation of the process of restitution of the polarized state. With the rest of the ventricle repolarizing at its normal rate, a new potential difference is thus established which will wax and wane during this phase of the heart cycle in the manner which the contour of the coronary T wave depicts.

Apparently, then, injury can cause not only 1, an alteration in the pattern in which the impulse spreads; 2, an injury current during rest, and 3, a lack of response of some regions, but in addition *a retardation of the recovery process in a region capable of responding more or less normally.* When the last occurs, the characteristic T wave appears. It is of note that this not only occurs later than the S-T deviation but originates in a different way and in a different region.

SUMMARY

The electrical changes produced by an area of injury on the dog's ventricle were studied in two types of experiments. In the first type, a very small injured area was produced by pressure, and by means of a unipolar lead from this spot, it was found that as the injury is produced, the spot becomes negative when the heart is at rest and positive during complete cardiac activation, measured with respect to the potential of normal uninjured cardiac muscle. These changes disappear on recovery from

the injury. This observation confirms the recent results of Eyster, Meek, et al. (6).

In the second type of experiment, a small area of injury was produced by intramyocardial injection of 95 per cent alcohol, and, by means of unipolar leads, the potential changes with time were followed in the injured area and at points on the ventricles at different distances from the center of injury. The results were:

1. Changes in the contour of the QRS complex, indicating an alteration in the pattern of impulse spread, occurred in the injured area and later also in other areas outside this region.

2. A depression of the S-T level occurred, which was maximum in the injured region and became smaller out toward the periphery. These changes tended to disappear with time.

3. A coronary type of upright T wave appeared some time after the injury was produced, and was confined to the margin of the injury and a narrow region surrounding it. This T wave tended to disappear with time.

An explanation of the results of both types of experiment is offered on the basis of the classical membrane theory.

1. The change in contour of the QRS produced by injury is ascribed to alteration in the pattern of impulse spread.

2. The T-Q elevation and S-T depression are attributed to the production by injury of a region which is partially depolarized at rest and irresponsive duration activation.

3. The late appearance of the large upright "coronary" T wave is ascribed to the production outside the original area of injury of a partially injured region which responds normally to activation, but which lags temporarily behind normal tissue in the process of repolarization.

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UTILIZATION OF THE KETONE BODIES IN NORMAL ANIMALS AND IN THOSE WITH KETOSIS

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The capacity of normal tissues to utilize ketone bodies was studied by Marriot (1) and later by Snapper and Grünbaum (2). The observations of these workers indicate that it is quite high. Chaikoff and Soskin (3) showed that diacetic acid injected into the depancreatized dog after hepatectomy was utilized by the other tissues of the body. Embden and his co-workers (4) found that the perfused liver added ketone bodies to the perfusing blood. Mirsky (5) showed that the ketosis caused by anterior pituitary extract needed the liver for its production. These observations together with more recent work (6, 7) points to the liver as the site of formation of ketone bodies in ketogenic states. In such conditions what is the rôle played by the other tissues of the body? Do they have a diminished capacity to utilize these substances and hence allow them to accumulate in the body or do they use these bodies at a normal rate, the ketosis resulting from a greatly increased production of them by the liver? The work reported in this paper is concerned with these questions.

Little accurate work has been carried out on the rate of ketone utilization of normal tissues or of the tissues of animals in a state of "ketosis." Chaikoff and Soskin (3) made measurements on their depancreatized dogs. They injected diacetic acid into these animals after hepatectomy and nephrectomy and followed the blood ketones thereafter. The injection carried the blood ketones to levels above 100 mgm. per cent, from which there was usually a rapid drop in the course of 2 or 3 hours to around 10–30 mgm. per cent, where there was a tendency to level off. These results would seem to suggest that the utilization rate was very high at the high levels but not of any great magnitude at levels found in ordinary conditions of ketosis. The recent work of Dye and Chidsey (8) supports this conclusion. Blixenkrone-Moller (9) measured the ketone body utilization of muscle by perfusing the hind quarters of cats and found that both normal and diabetic tissues burned ketone bodies at similar high rates. The concentration of the ketone bodies in the perfusing fluid was quite high.

The aim of our work was to measure the ketone body utilization in animals in ketosis, and in normals, with the blood ketone body concentration at the low levels ordinarily observed in "ketosis" and with a minimal change from the normal physiological state of the animal. This we carried out by the method of arterio-venous differences which has been previously described (10). The most important advantage of the method is that the tissues are not affected by the experimental procedures, as is the case in methods such as perfusion or tissue slice procedure, in which drastic changes may be made in the condition or environment of the tissue. Also the concentration of the ketone bodies in the blood, which may be a factor in determining the rate of utilization, is kept at the natural levels.

TABLE 1

PREPARATION	ACETONE BODIES AS ACETONE		A-V DIFF. AS ACETONE	A-V DIFF.* AS ACETONE BODIES	A-V DIFF. OXYGEN	PER CENT TOTAL ME TABOLISM AS ACETONE BODY OXIDATION
	Arterial	Venous				
Diabetic rabbit	3.14	1.57	1.6	3.1		
Diabetic goat	3.15	2.40	0.8	1.5	6.40	23.4
Diabetic dog	6.75	6.25	0.5	1.0	9.43	10.6
Diabetic dog	11.5	9.5	2.0	4.0	2.00	200.0
Diabetic dog	13.6	11.9	1.7	3.2	10.75	29.9
Diabetic dog	24.1	22.0	2.1	4.3	5.50	78.0
Phloridzin rabbit	3.08	1.75	1.3	2.6	9.00	28.8
Phloridzin rabbit	3.20	2.20	1.0	1.9	3.50	54.3
Phloridzin dog	6.80	6.10	0.7	1.4	4.36	32.1
Phloridzin dog	7.90	6.80	1.1	2.1	5.99	35.0
Phloridzin dog	25.60	23.60	2.0	4.1	5.75	71.4
Phloridzin dog	29.75	27.45	2.3	4.7	5.75	81.6

* "Acetone bodies" are estimated by assuming that they are present as aceto-acetic acid (25 per cent) and β-hydroxybutyric acid (75 per cent).

The bloods were taken from the femoral vein and artery. The venous bloods were taken first. Nine cubic centimeter samples were collected, sufficient for both ketone body (11) and oxygen (Van Slyke manometric) determinations. Ketosis in the diabetic animals was brought about by withholding food and insulin. Phloridzin ketosis was produced by fasting and the daily injection of one gram of the drug in olive oil. The results are given in table 1.

In column 6 is given the per cent of total metabolism taken by acetone bodies assuming 1 mgm. of mixed ketone bodies requires 1 cc. oxygen for combustion. This column actually gives the fraction of oxygen consumed by ketone bodies. However the energy produced per cubic centimeter oxygen used in burning other foodstuffs in the tissues probably differs

little from that for the ketone bodies so that the figure given in column 6 would approximate the percentage of total metabolism produced by burning of ketone bodies. In one case (diabetic dog 3) the figure is impossibly high. This is obviously due to the very low oxygen A-V difference. This could be caused by a temporary rapid blood flow which would lower the oxygen A-V difference, but would hardly affect that for the ketone bodies since their concentration in the tissues would be little affected by sudden changes in blood flow and it is this concentration which is in equilibrium with the venous blood. The average for all values in column 6 excluding that of 200 per cent is 44.5 per cent. It is to be noted that these values tend to be higher when the blood ketone concentrations are higher, which supports previous work (8).

Utilization in normals. The utilization of normal animals was carried out by determining the rate at which sodium dl-β-hydroxybutyrate could

TABLE 2

ANIMAL	WEIGHT	TIME OF INJECTION	AMOUNT INJECTED	AMOUNT EXCRETED	KETONE BODIES, BLOOD MGM. PER CENT		UTILIZATION, PER KILO MIN.*
					Start	End	
	kgm.	*minutes*	*mgm.*	*mgm.*			*mgm.*
Rabbit 1	2.66	17	74	0.7	3.9	3.9	1.62
Rabbit 2	2.78	25	120	12.1	10.5	11.7	1.31
Rabbit 3	2.08	23	101		7.6	6.7	2.30
Dog 1A	13.3	16	256	1.3	5.3	4.85	1.39
Dog 1B	13.3	13.5	205		6.0	5.3	1.80

* In calculating these figures a correction for the change in concentration in the tissues was made. It was assumed that 70 per cent of the body weight was water diluting the ketone bodies in the case of the dog, and 50 per cent for the rabbit.

be injected intravenously into them without causing a change in the blood concentration. In such a steady state the rate of injection minus the kidney excretion represents the rate of utilization by the tissues. Rates satisfying such a requirement were first established by trial and error in preliminary experiments. Thereafter our procedure was as follows: we injected the solution of sodium dl-β-hydroxybutyrate (0.4 per cent in saline) rapidly at first in order to bring the blood ketone level into the region of that of our "ketosis" animals and then injected at the steady rate we wished to study, taking blood samples for ketone determination at the beginning and end of the period. The results are given in table 2.

We may obtain an approximate idea of the utilization rates of our ketosis animals if we assume that the venous blood values are the same as those for the mixed venous blood. The utilization rates would then be the product of the A-V differences by the minute volume of the heart.

The values for our ketosis animals with low blood ketone levels are given in table 3. Cardiac outputs for the dog are from Marshall (12), for the goat from Barcroft et al. (13) and for the rabbit from Dock and Harrison (14). The utilization rates so estimated are if anything greater than in the controls.

The oxygen and ketone body arterio-venous differences were determined in some of the control animals and are given in table 4.

These differences on the average are smaller than those obtained on "ketosis" animals. This is probably due to the fact that the levels are rather low and also that but one of the ketone bodies was injected, whereas

TABLE 3

Ketone body utilization rates of "ketosis" animals which had blood ketone body levels in the same range as that of the control animals

ANIMAL	UTILIZATION, PER KILO MIN.
	mgm.
Diabetic goat	2.0
Diabetic dog	1.45
Phloridzin dog	2.03
Phloridzin dog	2.9
Diabetic rabbit	3.7
Phloridzin rabbit	3.1
Phloridzin rabbit	2.3

TABLE 4

ANIMAL	KETONE BODY DIFFERENCES	OXYGEN DIFFERENCE
	mgm. per cent	*cc. per cent*
Rabbit 2	1.0	2.2
Dog 1A	0.4	1.5
Dog 1B	1.2	5.5

the liver of the ketogenic animal adds both acetoacetic and β-hydroxybutyric acids to the blood. The liver is capable of changing β-hydroxybutyric acid to acetoacetic to some extent (15). There is nothing in these results that would indicate that the tissues of "ketosis" animals are any less able to utilize ketone bodies than normal animals. It seems hardly necessary to compare the capacities of the two types of animal at higher levels: the "ketosis" animals there obtain such a high percentage of their energy from ketone body burning that the normal could not possibly be much higher.

The rôle of ketone bodies in normal metabolism. A question arises when one considers that such a large fraction of total fat metabolism is carried

out through the intermediary steps of ketone bodies; is this a necessary preparation of the fats for the ready utilization of them by tissues like the muscles? It does not seem to be absolutely so since in fasting animals after hepatectomy (which stops all ketone body formation) there is good evidence of utilization of fat (16). The hepatectomized animal remains fairly quiet and has practically a basal metabolic rate. Would he be capable of carrying on a high metabolic rate as a result of exercise without the aid of this intermediary change of the fats if they were the predominant source of fuel? The utilization of ketone bodies by skeletal muscles is increased by having them work (9) although little change in ketone excretion or ketonemia level is brought about in the body as a whole as a result of exercise. It has been shown by one of us (17) that a subject on a constant ketogenic diet may tend to excrete somewhat more ketone bodies in the urine on days of inactivity than on days of large expenditures of energy. The differences are not large, however.[1]

TABLE 5

Blood ketone bodies in milligrams per cent

	1 DAY	2 DAY	3 DAY
8 a.m.	26.6	26.4	35.9
12 noon	10.3	9.5	8.8
3 p.m.		16.3	
Urine 8 a.m. to noon in mgm.	472	278	538

We made observations on a subject (75 kgm. wt.) on a constant ketogenic diet. On three successive days, with no breakfast, bloods were taken for ketone determinations at 8 a.m. and noon, and the urine was collected for the period. Between the time of the blood samples of the first morning the subject stayed in bed, on the second morning he engaged in heavy exercise and on the third he engaged in light exercise. On the second day after the noon blood was taken the subject continued without food but stopped the exercise, and at 3 p.m. a third blood was taken. The results are given in table 5.

The differences in urine excretion are relatively unimportant in comparison with the large amounts of ketone bodies which must have disappeared from the body in order to lower the blood level of them to such a degree. The heavy exercise of the second day did not cause any greater drop in blood ketone level than occurred in the control days. These results are not incompatible with the findings of Blixenkrone-Moller (9) which show that exercise increased the utilization rate of ketone bodies.

[1] Since this was written an article by Barker (J. Physiol., Vol. 97, No. 3) has appeared which reports similar findings.

These seemingly contradictory statements can be reconciled if we suppose that in "ketosis" states, the liver during exercise increases its production of ketone bodies commensurate with the increased utilization of them by the muscles and so leaves the balance between production and consumption the same as during rest. The blood ketone rise at 3 p.m. on the 2nd day of our series gives some support to this theory. We may best presume then that our present knowledge indicates that the ketone body intermediary transformation is not necessary for the combustion of fats but may be a supplementary mechanism operating particularly when the body must expend large amounts of energy with fat as the fuel.

Discussion. Our findings give no support to the concept that one molecule of fatty acid can give rise to but one molecule of ketone. The results indicate that from 30 to 80 per cent of the energy requirements of the tissues in ketogenic states may be supplied by combustion of ketone bodies. In the conditions produced, around 80 per cent of the ultimate source of energy was fat. This would be made up predominantly of long chain fatty acids, and one cannot account for such a large ketone body production and utilization if each molecule of fatty acid gave rise to but one molecule of ketone body. Butts (18), Deuel (19) and their co-workers using rat feeding experiments have shown that the urinary acetone body production is greater than can be accounted for by a one to one ratio. Blixenkrone-Moller (20) from observations on perfused diabetic cat livers concludes that 4 molecules of ketones may be formed per molecule of fatty acid. The recent work of Stadie, Laff and Lukens (21) with liver slices supports such a view. We have therefore abundant support in the literature for the higher than one to one ratio between fatty acid and ketone which is necessary to explain our results.

SUMMARY

We have determined the ketone body utilization of animals in a state of ketosis caused by pancreatic diabetes or phloridzin. The method used was that of simultaneous ketone body and oxygen arterio-venous differences. The average fraction of the oxygen difference utilized by burning ketone bodies was 44 per cent.

Normal animals injected with β-hydroxybutyric acid show no greater utilization of ketone bodies than animals with ketosis.

We have presented the view that the production of ketone bodies by the liver and utilization of them by the other tissues is an important, though not necessarily inevitable, route for the catabolism of fatty acids. When the organism is in a state of ketosis, increases in metabolic rate (as in exercise) probably increase the rates of production and utilization of these substances.

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THE CAPACITY FOR VIGOROUS MUSCULAR ACTIVITY OF NORMAL RATS AND OF RATS AFTER REMOVAL OF THE ADRENAL MEDULLA

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The bulk of the experimental evidence on the function of the adrenal medulla seems to indicate that this portion of the gland plays no significant rôle in bodily economy during periods of rest, but that in conditions of emotion, asphyxia, exposure to cold and violent muscular exercise epinephrine is liberated reflexly. Functional changes following the injection of epinephrine and those following stimulation of the sympathetic nervous system are similar and Cannon has pointed out that these functional responses are useful in preparing the animal for activity in conditions of stress. The effects observed when epinephrine is injected include acceleration of the heart and augmentation of the heart beat, constriction of the blood vessels of the skin and splanchnic regions, increased blood pressure, relaxation of the bronchioles, deepened respiration, glycogenolysis in the liver, hyperglycemia, and release of erythrocytes from the spleen. All of these are valuable physiologic reinforcements in situations in which intense muscular activity is demanded. The functional importance of the adreno-sympathetic mechanism in mediating these important physiologic responses during emergencies is well established. Studies on animals in which the adrenal medulla has been destroyed by a method which does not limit the functional activity of the adrenal cortex, and in which the rest of the sympathetic system has been left intact, however, have given little convincing evidence that animals following destruction of the adrenal medulla are less capable of survival than a normal animal in a condition of stress which involves vigorous muscular activity.

Ingle, Hales and Haslerud found that destruction of the adrenal medulla did not limit the capacity of the rat to continue the work of the stimulated gastrocnemius muscle. In a recent study, one of us (D. J. I.) (4) observed that during the first few hours of stimulation the height of muscular contraction is maintained much better by normal rats than by rats from

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which the adrenal medulla has been removed. However, after a few hours the amounts of work performed became similar and the total amount of work performed before the loss of muscular responsiveness by rats after destruction of the adrenal medulla was just as great as that of normal rats. Ingle and Harris observed that the voluntary activity of rats after destruction of the adrenal medulla was normal. Richter confirmed this observation. Campos, Cannon, Lundin and Walker found that inactivation of the adrenal medulla of the dog did not limit its capacity for prolonged work on the treadmill.

In the present study we have compared the time taken for normal rats and for rats after removal of the adrenal medulla to swim until exhausted. The "emergency" type of situation described by Cannon is more nearly approximated than in previous experiments.

METHODS. The adrenal glands of male rats of the Wistar strain were enucleated by technic described by Evans. The operations were performed when the animals weighed from 45 to 85 grams. Similar rats had incisions only. All operations were performed with the animals under ether anesthesia and sterile technic was used.

The tank used for swimming was made of galvanized iron, 22 inches (55.5 cm.) deep and 17 inches (42.5 cm.) in diameter. It was filled to a uniform depth for each series of tests with water held at a constant temperature of 30°C. All the tests were made at the same time of day. The animals were fasted for twenty-four hours before the tests. Since rats are capable of swimming continuously for several hours before exhaustion the time was shortened by tying a weight close to the proximal end of the tail. With a weight of 10 grams the average time required for exhaustion was from ten to fifteen minutes; with a weight of 20 grams, two to three minutes. The experimenter (R. E. H.) who made the observations on swimming time was not aware of the identity of the animals at the time the tests were made.

EXPERIMENTS AND RESULTS. A preliminary experiment was performed in which a group of rats which had been used in previous experiments swam with 20 gram weights attached to their tails. These animals were heterogeneous in body weight. Twenty-five rats in which the adrenal medulla had been destroyed were compared to twenty-three animals which had been subjected to incision only. There was no significant difference in the average time required for exhaustion in the two groups.

In experiment 1, the animals were forced to swim when they reached a body weight of 180 grams with a weight of 20 grams. There was no significant difference in the average time required for the rats in the two groups to swim to the point of exhaustion. In experiment 2, the animals were weighted with 10 grams, thus lengthening the time required for exhaustion. The rats without adrenal medullas were superior to their con-

trols, this time to a point approaching the usual criterion for statistical significance. Since there is no obvious reason for the differences in favor of the animals without adrenal medullas, we are inclined to attribute the difference found to the vagaries of small sample in an undefined but probably homogeneous population.

Twenty of these animals were retested later, not less than two weeks after the first test. The coefficient of correlation between the scores on the test and retest was $+0.90 \pm 0.03$. The results of experiments 1 and 2 are presented in table 1.

COMMENT. It is reasonable to conclude that under these experimental conditions the absence of the adrenal medulla does not decrease the capacity of the rat to respond in a normal manner to this type of "emergency" situation. To generalize beyond these experimental conditions to the rôle of the adrenal medulla in all conditions of stress is not justifiable. It was not established by these experiments that there is a reflex discharge

TABLE 1

Time required to swim to point of exhaustion: rats with and without adrenal medullas

EXPERIMENT	ADRENAL MEDULLA	NUMBER OF RATS	WEIGHT ATTACHED	AVERAGE TIME	DIFFERENCE	FISHER'S t*
			grams	*seconds*	*seconds*	
1	Removed	28	20	137.25		
	Not removed	28	20	133.86	3.39	0.404
2	Removed	27	10	719.32		
	Not removed	23	10	597.71	121.61	2.04

* Fisher, R. A. Statistical methods for research workers. Edinburgh, Oliver & Boyd, 1930, p. 108.

of epinephrine from the adrenal medulla during vigorous swimming. Unfortunately, there is no satisfactory test for the presence of epinephrine in the blood of the rat. Moreover, there are other possible sources of epinephrine or epinephrine-like substances which are not eliminated by the destruction of the adrenal medulla.

SUMMARY

Normal rats and rats after removal of the adrenal medulla were compared in respect to the times required to swim to exhaustion when they were handicapped by weighting. The performances of rats without the adrenal medullas were as good under these experimental conditions as those of normal rats.

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THE INTERACTION OF CENTRAL AND PERIPHERAL CHEMICAL CONTROL OF BREATHING[1]

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Before the chemoceptive function of the carotid and aortic bodies was known, the chemical control of breathing seemed a relatively simple problem. Lack of O_2 and excess of CO_2 were regarded as normal respiratory stimuli operating solely at the respiratory center, and the changes in pulmonary ventilation of such chemical origin appeared to harmonize with changes in acidity of the respiratory center (Gesell, 1925, 1929). But when reflexogenic chemical control (Heymans, Bouckaert and Dautrebande, 1930) as well as centrogenic control of breathing was established new problems developed. Not only was it desirable to know the relative parts played by centrogenic and reflexogenic control, and the response of the center and of the chemoceptor to O_2 lack and CO_2 excess, but it was of equal interest to determine the interaction of the central and peripheral mechanisms. Our present experiments bear on these fundamental issues and we believe offer a simple reconcilation of facts with the acid mechanism of control.

METHOD. Our method was relatively simple. It consisted essentially of temporary bilateral blocking and deblocking of Hering's nerve during normal and modified breathing. The vagus nerves were sectioned to permanently eliminate those chemoceptor signals arising in the aortic bodies and to abolish interfering pressure reflexes arising in the aortic arch. Hering nerve block, therefore, prevented all known remaining chemoceptive signals from reaching the center and thus revealed breathing of purely centrogenic origin. Deblocking returned the reflexogenic component.

The cold blocks were made of copper, shaped to fit neatly into the region of the nerve after removal of the larynx. They were chilled and warmed with rapidly circulating alcohol. Temperature changes between 37°C and −3°C required 30 seconds. The moment of blocking and deblocking was signaled when the temperature reached 0°C and 30°C respectively.

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[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

In preliminary experiments it was found that temporary Hering nerve block during eupnea might produce one of three effects,—increased breathing, decreased breathing, or no observable change. Such variability of results, noted by others as well (see Stella, 1935), is readily explained by the simultaneous elimination of two sets of signals ascending Hering's nerve: *a*, excitatory signals coming from actively discharging chemoceptors of the carotid bodies, and *b*, inhibitory signals coming from stretched endings of the carotid sinuses. Should the inhibitory action of the carotid sinus be greater than the excitatory action of the carotid body an increased volume of breathing would be expected to occur during nerve block. On the other hand, should the excitatory action of the carotid body be greater than the inhibitory action of the carotid sinus decreased breathing would occur. Should both actions be equal there would be no change in breathing at all.

The correctness of these assumptions was confirmed by further orientation experiments in which blood pressure changes were automatically compensated at relatively low pressure levels or in which the carotid sinuses were collapsed. Under these circumstances block never produced an increase of breathing. There was either a diminution or no effect at all. Each sinus was, therefore, routinely collapsed.[3]

Our observations were made exclusively on dogs (anesthetized with morphine (3.5 mgm/kilo) and chloralose (100 mgm/kilo)) under the following conditions:

1. During hypocapnia produced by excessive artificial ventilation with a room air mixture.

2. During acute hypercapnia produced by the administration of 10 and 15 per cent CO_2 mixtures in 65 per cent O_2.

3. During progressive hypercapnia produced by rebreathing a small volume of a high O_2 mixture without reabsorbing the exhaled CO_2.

4. During acute O_2 lack of graded intensities produced by breathing varying O_2 mixtures in N_2 (40 to 6 per cent O_2 in N_2).

Centrogenic and reflexogenic breathing in the eupneic range of chemical

[3] The sinuses were relieved of their normal distention by tying one ligature around the common carotid artery approximately an inch below the sinus, a second around the internal carotid just distal to the sinus and a third around the external carotid above and as close to the sinus as possible (Gollwitzer-Meier, 1934) and then puncturing the common carotid peripheral to ligature 1. As Winder (1933) and Winder, Bernthal and Weeks (1938) point out, most effective anastomoses act to preserve a uniform flow of blood through the carotid body on occluding the common carotid artery. A concomitant rise in systemic pressure from elimination of the sino depressor reflex acting through the circle of Willis would tend to maintain a uniform head of pressure in the occipital artery supplying the carotid body. In that event our results would give a fairly reliable indication of the relation rôle of the chemoceptors during eupnea.

stimulation. The capacity of the center and chemoceptor to respond to the major respiratory stimuli (i.e., O_2 lack or CO_2 excess) is now accepted as fact. (For references see Heymans and Bouckaert, 1939; Gesell, 1939; and Schmidt and Comroe, 1940.) The similarity of the respiratory tracing during CO_2 administration, before and after chemoceptive denervation, leaves no doubt of the capacity of the center to respond to chemical changes occurring within itself. The smallness or the absence of response to O_2 deficiency after chemoceptive denervation shows the effectiveness of reflexogenic breathing. Despite the general agreement on this point considerable discussion still remains regarding the relative effectiveness of CO_2 excess and O_2 deficiency at the center and chemoceptor respectively. Heymans and his associates (1939) insist on the predominating rôle of the carotid body, for CO_2 as well as O_2 regulation. This view was supported by the intense hyperpnea which they and others produced by a localized hypercapnia in the vascularly isolated carotid body. They pointed to the significant observation that this hyperpnea persisted despite an undoubted overventilation and an hypocapnic condition of the respiratory center.

Comroe and Schmidt (1938), Schmidt and Comroe (1940) and Schmidt, Dumke and Dripps (1939), however, arrive at opposite results and conclude 1, that the vascularly isolated carotid body exhibits a low reactivity to changes of arterial carbon dioxide and oxygen; 2, that denervation of the carotid and aortic bodies produces no uniform effect upon alveolar carbon dioxide and, therefore, has no important effect upon eupneic breathing, and 3, that denervation neither retards nor diminishes the respiratory response to carbon dioxide. In their opinion "Carotid body reflexes constitute an accessory mechanism, brought into action by emergencies such as foreign chemicals, anoxemia, and unusually great increases in the CO_2 tension of the blood, rather than an essential part of the normal respiratory regulating system; the control of breathing under ordinary conditions is accomplished entirely by the direct effects of chemical stimuli (mainly CO_2) upon the cells of the center." The denervation experiments of von Euler and Liljestrand (1936) differ in turn from those of Schmidt and Comroe. They found an increased alveolar CO_2 pressure after denervation during eupnea and interpreted this change as a sign of diminished breathing. Bernthal and Weeks (1939) found that breathing and vasomotor activity were reduced when the carotid bodies were cooled. Bogue and Stella (1935), Samaan and Stella (1935) and von Euler, Liljestrand and Zotterman (1939) found low CO_2 thresholds for activation of the carotid body and Bernthal (1938) found a reaction to small changes in carbon dioxide pressures. These results must be interpreted to mean that the center and the chemoceptors participate jointly in the control of eupneic breathing and that a higher intensity of chemical stimulation

is required to drive the respiratory machine when the chemoceptors are out of function. Our own orientation experiments already cited indicate the same for at least a portion of the animals.

The central response to oxygen deficiency is either missing or decidedly diminished when tested under anesthesia (see reviews of Heymans and Bouckaert, 1939; Gesell, 1939; Schmidt and Comroe, 1940) and, therefore, must be of little practical value to the animal. Reflexogenic breathing is without doubt the important component under such conditions. Only in the absence of anesthesia is central hyperpnea said to approach hyperpnea in the intact animal, (Dautrebande, 1939) a finding denied by Bouckaert, Heymans and Samaan (1938). While Schmidt and Comroe (1938) find a relatively high threshold for anoxemia in the carotid body preparations, Bernthal (1938) and von Euler, Liljestrand and Zotterman (1939) find the threshold within the eupneic range of oxygen pressure.

Our methods under conditions 1, (hypocapnia from overventilation) yield further information on the relative effectiveness of centrogenic and reflexogenic breathing during eupnea with a slightly different procedure. Dogs were connected with rebreathing tanks containing room air. Respiratory stimulation was then diminished by artificial overventilation of the lungs, sufficient to reduce or stop natural breathing after artificial ventilation was ended. As soon as standard conditions yielding a dependably uniform series of apneas or subnormal respiration had been established, Hering's nerves were blocked at the end of every second period of artificial ventilation. They were deblocked after natural breathing had returned. In the first of the two experiments used to illustrate our results, the respiratory tracing is seen to begin in eupnea and was presently followed by two minutes of artificial ventilation (see upper record). As indicated by the horizontal bar, nerve block began about one minute before the end of artificial ventilation and deblocking occurred shortly after the end of apnea. Whenever the chemoceptor signals were blocked in this experiment nearly one minute was required to rebuild a stimulus strong enough to interrupt the apnea produced by overventilation. But when the centrogenic and reflexogenic components were allowed to complement each other, breathing started immediately after cessation of ventilation. In the second experiment, in which overventilation was more effective (see records 2 and 3 of fig. 1) the duration of apnea was increased threefold whenever Hering's nerves were blocked. This was interpreted to mean that the threshold stimulus required to reinitiate breathing after the production of apnea is lower for the intact respiratory mechanism than for the center alone, working without the aid of the chemoceptor signals.

It seems most significant that the rôle of the chemoceptors should be so strikingly revealed in the duration of apnea when their influence upon the depth of eupneic breathing is disproportionately less (see the effects of

blocking and deblocking in the lower record). For that reason the shortening of apnea might readily be interpreted as a different phenomenon from that of the tonic stimulation of the center. But if alveolar oxygen pressures fell more precipitously than carbon dioxide pressures rose during apnea it is probable that breathing was reinitiated at a moment when both oxygen and carbon dioxide pressures were below eupneic levels, as happened in Haldane's experiments on man (1922). Oxygen lack, thereby, becomes the logical initiator of breathing in our experiments on

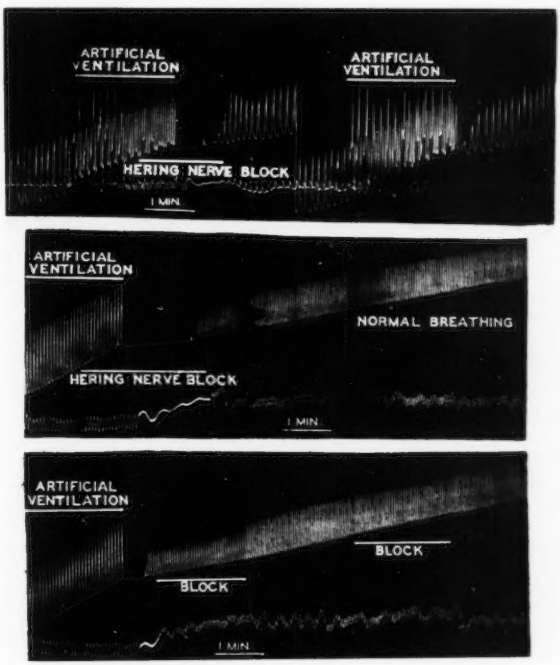

Fig. 1. Prolongation of apnea by withdrawal of reflexogenic support

the dog as well as in those on man. But in reaching this conclusion it is essential to remember that the effects of oxygen and carbon dioxide cannot possibly be separated if cH is the common stimulus to both. Lack of oxygen which leads to lactic acid formation must of necessity decrease the buffer base and increase the effectiveness of the prevailing carbon dioxide pressures. Theory, therefore, demands that the subeupneic carbon dioxide pressures at the moment of reinitiation of breathing contribute towards the stimulation of oxygen lack. Haldane's belief that lack of oxygen in some way increases the excitability of the center to carbon

dioxide agrees with this conception. This reasoning implies great responsibility of the chemoceptors as tonic controllers of oxygen pressures within the normal range of physiological stimulation.

Hypercapnia. The effects of high concentrations of carbon dioxide before and after deafferentation of the carotid and aortic bodies have already been described. The fact that only one comparison is possible upon a single animal makes a quantitative analysis of the relative importance of centrogenic and reflexogenic breathing difficult. This disadvantage is overcome in our experiments. Dogs were connected, as usual, with rebreathing tanks containing high carbon dioxide mixtures (10 to 15 per cent CO_2 in 65 per cent O_2 in N_2) and after hyperpnea had been well established Hering's nerves were alternately blocked and deblocked. The nerves remained unmolested in blocking position during comparative observations which eliminated the possibility of mechanical disturbance. Though the continuity of recording permitted a detection of the smallest changes in pulmonary ventilation, at no time did we notice a change in either the depth or frequency of the hyperpnea on nerve block. Subsequent tests with low O_2 or cyanide showed that the nerves must have been in good condition. Whatever the interpretation of our results may be, we see for the moment that the findings are not in complete accord with a predominant rôle ascribed to the chemoceptors in CO_2 control by Heymans. On the other hand if 10 or 15 per cent CO_2 in the inspired air can be regarded as an "emergency" they are in no better agreement with the position of Schmidt and Comroe whose schematic representation of centrogenic and reflexogenic breathing shows a powerful peripheral stimulation at high CO_2 pressures (1940).

This lack of reflexogenic breathing at high CO_2 pressures is in accord with the general evidence from many groups of experiments mentioned above on hypercapnia, before and after denervation. It is puzzling in face of the common findings that local carotid body activity actually does increase with increasing CO_2 pressures as is so very clearly indicated by the increased breathing produced by *localized* hypercapnia and by the linear relation of frequency of chemoceptive impulses to CO_2 pressures, ranging up to 14 per cent in the inspired air (von Euler, Liljestrand and Zotterman, 1939). Could it be that a generalized hypercapnia abolished in some way the central action of the signals which CO_2 set up in the periphery? And if this were true, at what pressures does the reflexogenic component fall out? These questions were studied by administering a 65 per cent O_2 mixture in N_2 with the aid of a rebreathing tank and allowing a rapid accumulation of the expired CO_2 in a limited volume of gas. Hering's nerves were blocked and deblocked and gaseous samples extracted from the tanks at appropriate intervals. Reference to figure 2 reveals the type of results obtained. It will be seen at once that breathing was

reduced by nerve block at the lower CO_2 pressures (expressed in per cent of CO_2 in the inspired O_2 mixtures) but not at the higher pressures and that the effect of blocking was no longer noticeable when the CO_2 in the inspired air had increased to approximately 5 to 6 per cent. We have, therefore, arrived at a rather paradoxical conclusion regarding the rôle of the carotid bodies. As physiological controllers of CO_2 pressures they are least effective when subjected to powerful stimulation and most effective when subjected to weak stimulation.

An explanation of the vanishing reflexogenic component was suggested by the earlier experiments of Gesell and Moyer (1935) in which hypercapnia was found to reduce or abolish respiratory reflexes, such as retardation and acceleration of breathing produced by central stimulation of the vagus and saphenous nerves respectively. Does carbon dioxide also abolish the central action of the very signals which it sets up in the carotid bodies? We believe it does.

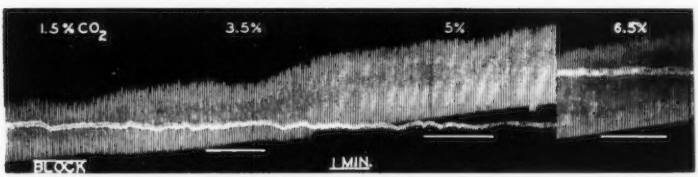

Fig. 2. Effects of blocking chemoceptor signals on pulmonary ventilation during progressively increasing hypercapnia.

Though our findings are incompatible with a predominant rôle ascribed to the carotid bodies by Heymans when carbon dioxide pressures are high they agree better with his views when the pressures are low. They fail, however, to harmonize with the views of Schmidt and Comroe at either high or low carbon dioxide pressures. According to their statement and curves (1940) they "actually found" an increasing reflexogenic component which at high carbon dioxide pressures was greater than the centrogenic component. So far as we are aware no confirmation of such results exists in the literature and our results indicate a progressively decreasing reflexogenic component replaced by an increasing centrogenic component as carbon dioxide pressure increases. The curves of Schmidt and Comroe were probably compiled from data taken during localized carotid body hypercapnia without consideration of the functioning of the respiratory mechanism as a whole.

Oxygen lack. Typical effects of O_2 lack on centrogenic and reflexogenic breathing are shown in the nerve block tests of figure 3 in which a dog successively breathed five mixtures of oxygen in nitrogen (40.0 per cent, 19.7 per cent, 16.6 per cent, 12.5 per cent and 8.6 per cent). The tests

began with a high oxygen mixture and showed the diminished breathing which so often occurred during Hering nerve block when the arterial blood was supposedly saturated with oxygen. Granting an absence of ischemia in the carotid bodies, the centrogenic breathing which remained and the reflexogenic breathing which was removed by block were probably the result of the stimulating action of CO_2. The effects of oxygen

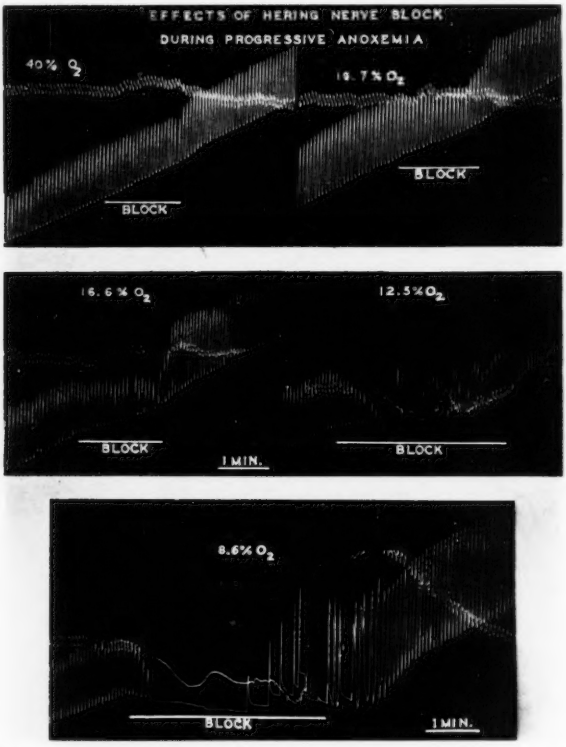

Fig. 3. Effects of withdrawing reflexogenic support during progressively increasing hypoöxia.

lack were as readily demonstrated when the oxygen of the inspired air was lowered. It will be seen that when the dog was simply switched from the 40 per cent O_2 mixture to the 19.7 per cent O_2 mixture, breathing was augmented. This in itself is indicative of a sensitive response of the respiratory mechanism to a slight reduction of arterial oxygen pressure for hyperventilation of this kind is always associated with a marked drop in carbon dioxide pressures. That the respiratory stimulation actually

occurred in the chemoceptor is seen in the large reduction of breathing which occurred when the chemoceptor signals were blocked (compare the results of the first and second blocks). Since the centrogenic breathing in the second observation was less than in the first, reflexogenic breathing must have been increased by the oxygen deficiency. The hyperpnea, which prevailed before nerve block, occurred despite a diminished central support.

This diminished central support became more marked as rebreathing continued and oxygen want increased. In the experiment under consideration it reached its limits in the prolonged apnea when the oxygen in the inspired air stood at 8.6 per cent. Such diminishing central support is a most significant phenomenon in relation to the chemical mechanism of respiratory control. It is conceivably due to two causes. One is the ultimate paralyzing action of oxygen lack. The other is the alkalinizing effects related to increased elimination of CO_2 from increased ventilation of the lungs and blood, increased volume flow of blood, and increased CO_2 carrying capacity and pH of the blood. As the alkalinizing influence of oxygen deficiency increases, either from an increasing ventilation or from a long continuance of hyperpnea, centrogenic stimulation would decrease in proportion. Therefore, successive Hering nerve blocks would be expected to reveal a decreasing magnitude of centrogenic breathing. This view was expressed some years ago (Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932) on the basis of direct measurement of the amount of CO_2 eliminated and of the increase of the respiratory quotient during anoxemia.

An acid interpretation of the diminishing centrogenic breathing during oxygen deficiency is of course tenable only on the assumption that the so called central "paralysis" is not an important factor. The sudden increase of pulmonary ventilation occurring at the moment of deblocking of Hering's nerves indicated a fitness of the centers for they responded immediately to the burst of signals released from the carotid bodies. The sudden and pronounced acceleration of breathing produced by deblocking of the vagus nerves (not illustrated) showed a similar fitness of the centers to react to proprioceptive signals. We are, therefore, inclined to believe that the apneas noted at low oxygen pressures were not paralytic, that the hyperpneas were reflexogenic and occurred despite a condition of central hypocapnic apnea. In this connection it is well to recall that hyperpnea and central acapnia are not incompatible. As Gesell and Moyer (1935) showed, a center made apneic by the injection of Na_2CO_3 is more highly responsive to central stimulation of the saphenous nerve.

But the prolonged apnea (at 8.6 per cent O_2) was eventually broken in the absence of any known change of peripheral stimuli. Control experiments showed that the renewed breathing cannot be alternately explained

by an accidental incomplete block permitting conduction of chemoceptive signals at the peak of a heightened carotid body discharge, because the same type of renewed breathing occurred after bilateral distal section of the sinus nerves during the apnea of cold block. Provided unknown reflexogenic stimulation from sources other than the carotid and aortic bodies can be disregarded, the renewed breathing must be considered of centrogenic origin. For the present, the nature of the stimulus reinitiating breathing can only be conjectured by a process of elimination. Had the apnea been caused entirely by a paralyzing action, that action would have been expected to increase and to have terminated in death. Had the stimulation of breathing been one of *direct* action of O_2 lack, there should have been supernormal rather than subnormal centrogenic breathing when cold block took effect. But if the apnea was due to acapnia, time was essential for a reaccumulation of acid and a rebuilding of the central stimulus. We suggest that this occurred partly as a result of the high anaerobic acid metabolism in the brain and partly as an effect of the reaccumulating acid in the blood.

For completeness it must be mentioned that apneas frequently did terminate in death without outward signs of respiratory stimulation. It is, therefore, reasonable to assume that depression capable of completely counteracting stimulation can and does occur. Signs of such depression are visible in the falling blood pressure during the last two nerve blocks of figure 3.

Discussion. *The summation of centrogenic and reflexogenic breathing.* One point seems clear from the experimental findings of other laboratories, and those which we have described. Eupneic breathing in anesthetized animals is a sum of the two respiratory components—centrogenic breathing plus reflexogenic breathing. But, so far as we are aware, there has been no attempt to establish a mechanism by which they are combined. Some common denominator must, therefore, be found to account for the complementary action between centrogenic and reflexogenic breathing. The inherent forces arising in the neuron proper and those forces arising from the impingement of signals at the synapse must in some way be combined.

The electrotonic theory of nerve cell discharge and *synaptic drive* (Gesell, 1939, 1940) lends itself to such speculation and offers a relatively simple schema. (See figs. 4 and 5.) Due to a steep metabolic gradient between the dendrites and the axon hillock, estimated as 10 to 1, (Holmes, 1932) an electrotonic current is conceived to flow within the cell from the dendrites to the axon hillock. Because of the high lineal resistance of the neuraxon, the current is deflected at the axon hillock where it leaves the cell body, to return in the immediate external environment, back to the dendrites. On leaving the axon hillock, it is thought to fire this structure

at a frequency proportional to the intensity of the electronic current. Metabolic physico-chemical fluctuations, such as result from changes in O_2 and CO_2, are thought in turn to modify the intensity of this current. Changing intensity of and changing response to the electrotonic current would thus represent our so called "centrogenic component" of respiratory control.

Each of these cells (probably the reticular cells of the medulla) is covered with a dense layer of hundreds or thousands of synapses, delivering signals from all quarters, including the chemoceptors. Each signal, regardless of its origin, is thought to produce a local negativity at its point

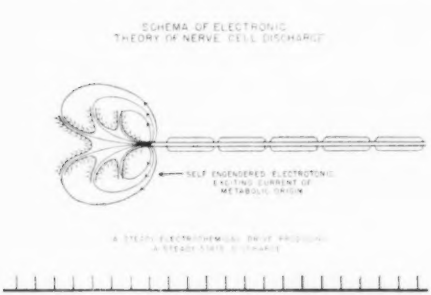

Fig. 4

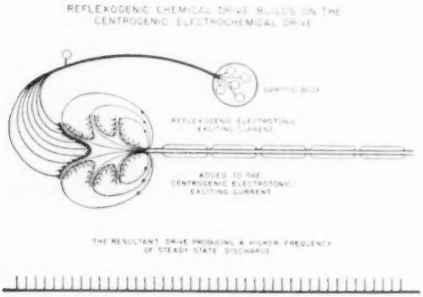

Fig. 5

Figs. 4 and 5. Schema showing a nervous mechanism for the summation of centrogenic and reflexogenic breathing.

of impingement and thereby increase the potential drop of the receiving neuron.[4] The intensity of the reflexogenic drive (or the reflexogenic component of breathing) is accordingly determined by the sum total of signals arriving per unit of time. Complementary action of the centrogenic and reflexogenic components thus becomes a simple matter of the addition or subtraction of one current to or from the other. The interaction of this dual mechanism of nerve cell activation allows not only a change in the sum total of centrogenic and reflexogenic components but gross differences in the relative proportions. At one extreme in which

[4] This hypothetical negativity may conceivably arise from either a specific activation or from an increased dendritic metabolism, initiated by a local electrical discharge or a chemical deposition at the synapse. Both could increase the metabolic or potential gradient and thereby the nerve cell discharge. The fact that breathing diminished gradually during the course of a continued Hering nerve block (see the lower record of fig. 1 and all of the records of fig. 3) suggests that synaptic effects long outlast the moment of their initiation. This might be regarded as a new interpretation of the general phenomenon of "after discharge." More specifically the results suggest that chemoceptor signals help to maintain the respiratory neurons at a higher degree of reactivity.

central apnea is produced by excessive pulmonary ventilation during oxygen deficiency, the central neurons would still retain their ability to respond to increasing reflexogenic electrotonic current even though the centrogenic electrotonic current is weakening. And this condition can shift to the other extreme of hypercapnia in which centrogenic breathing continues to increase long after reflexogenic breathing is abolished (see fig. 6).

The existence of two mechanisms of respiratory control, one central and the other peripheral, carries most interesting implications. Since both mechanisms seem to react to the common stimulus of cH, both may be expected to participate in the control of CO_2 and O_2 pressures in the

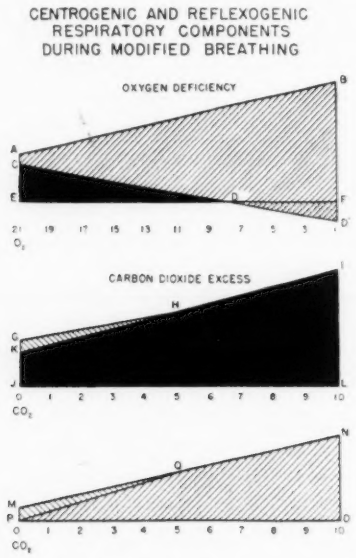

Fig. 6. Schema showing the oppositely changing proportions of centrogenic and reflexogenic breathing in progressively increasing hypoöxia and hypercapnia.

body. Nevertheless hyperpnea, caused either by oxygen scarcity, or carbon dioxide excess, tends to become exclusively reflexogenic or centrogenic. In other words, one mechanism gains the upper hand of the other and maintains primary control. This paradoxical situation, we believe, is explainable with the aid of the reaction theory. During O_2 lack the chemoceptors by virtue of a disproportionately high reactivity to changes in their own acid metabolism (Winder, 1937; Bernthal, 1938; Bernthal and Weeks, 1939; Winder, Bernthal and Weeks, 1938; von Euler, Liljestrand and Zotterman, 1939) gain the advantage and give increasing predominance to reflexogenic breathing as scarcity of oxygen grows. As

a result of increasing ventilation and of the other alkalinizing influences, centrogenic breathing is diminished. We have attempted to indicate these trends of centrogenic and reflexogenic breathing under theoretically ideal conditions in figure 6 in which so called central paralysis is missing. Both types of breathing are plotted on the ordinates against oxygen percentage of the inspired air on the abscissas. The solid black area *ECD* represents the centrogenic component, rapidly diminishing as a result of increasing hypocapnia, and the cross hatched area *ABFD'DC*, the more rapidly increasing reflexogenic component, possibly potentiated by synaptic alkalinization. The stippled area *DFD'* indicates diminution of the subliminal centrogenic component.

According to this conception the chemoceptors are not fully protected against increasing acidity by the increased ventilation which they set up. They alone withstand increased acidity and thus guard the more delicate central nervous system. This will explain why it was unreasonable to expect an increased amount of lactic acid in the circulating blood of an individual exposed to low O_2 pressures. The amount of lactic acid contributed to the circulating blood by approximately one millionth of the body could not possibly be detected.

The diametrically opposite changes in centrogenic and reflexogenic breathing during progressive hypercapnia must have some deep rooted significance (see two lower schema of fig. 6). *GJ* represents the volume of eupneic breathing of which *KJ* is the centrogenic fraction produced by the stimulating action of CO_2 and *KG* the reflexogenic fraction. In agreement with the linear relation of the discharge frequency of the carotid body to the prevailing CO_2 pressures (von Euler, Liljestrand and Zotterman) we may assume an hypothetical reflexogenic breathing increasing along the gradient *MN* of the lower graph. The area *MNOP* would accordingly represent the theoretical increase of reflexogenic breathing with increasing hypercapnia. The actual amount of reflexogenic breathing, however, is represented by area *MQP* or *GHK* above. It is, therefore, proposed that most of the reflexogenic component *PQNO* is obliterated by a central action of CO_2, possibly by a blocking action at the synapse. As this obliteration progresses, direct central stimulation replaces that lost from the chemoceptors. Whether *KI* (centrogenic increase) runs more steeply than *MN* (reflexogenic increase) has not been determined. These graphs are of course schematic. However, one cannot avoid the question at this point, why teleologically the center takes complete control against CO_2 excesses when the chemoceptors take complete responsibility during oxygen deficiencies. The evolutionary forces which were responsible for this unique arrangement can only be conjectured. The brain is well known to require a uniformly abundant supply of oxygen while on the other hand it tolerates high pressures of CO_2 with relative impunity.

Outlying protection against the development of central oxygen deficiency is, therefore, useful. On the other hand the weakening of respiratory reflexes by a general hypercapnia may have been the issue forcing the evolution of a centrogenic mechanism of control against CO_2 excesses. A flood of carbon dioxide liberated in combat might otherwise have put an end to pulmonary ventilation when it was needed most.

SUMMARY AND CONCLUSIONS

Repeated withdrawal of known chemo-reflex support to the respiratory center (bilateral reversible cold blocking of Hering's nerve after double vagotomy and permanent sinus collapse in chloralosed dogs) during various respiratory states yielded data and conclusions as follows.

During eupneic breathing of atmospheric air or O_2 rich air, chemoceptive nerve block usually reduced the volume of pulmonary ventilation. The reduction was smaller with an O_2 rich mixture than with a mixture containing but slightly less O_2 than room air. Reasons were presented for concluding that both CO_2 and O_2 pressures prevailing during eupnea are sources of reflexogenic respiratory support.

Apnea produced by overventilation with room air was markedly prolonged by chemoceptive nerve block. This effect was much greater than the reduction of breathing by chemoceptive block during eupnea. It was concluded that the chemoceptors exert an important tonic stimulation of breathing and that they are particularly responsive to oxygen lack occurring at the end of apnea.

Repeated withdrawal of reflexogenic support during progressive hypercapnia caused a diminishing absolute reduction in breathing which disappeared at 5 to 6 per cent CO_2 in the inspired air. It was concluded that hyperpnea of high grade hypercapnia is purely centrogenic.

The peculiar absence of reflexogenic stimulation could not be explained by central paralysis, for pulmonary ventilation continued to increase with increasing CO_2 well above the 6 per cent level.

In view of the linear relation of chemoceptor discharge to CO_2 pressure, and of the progressively increasing centrogenic activity in these experiments, it is concluded that increasing CO_2 exerts an increasing central blocking action on the signals which it sets up in the chemoceptors.

Conversely, diminishing pressures are thought to diminish the central blocking action of CO_2 and, thereby, potentiate the signals arising in the chemoceptors. This relationship will explain the stimulating action of low CO_2 pressures obtaining during eupnea, and at the end of experimental apneas.

Repeated chemoceptive nerve blocks during progressively increasing hypoöxic hyperpnea produced progressively increasing reduction of breathing, vigorous breathing being finally converted to apnea. It is

concluded that hyperpnea of high grade O_2 deficiency is purely reflexogenic.

In view of the abrupt resumption of hyperpnea on chemoceptive deblock and of the suddenly increased frequency of breathing on vagal deblock, it is concluded that central depression or paralysis was but a minor factor in the reduction of the centrogenic component and that progressive hypocapnea and alkalinization from several causes was a major factor in the diminishing centrogenic component. It is further proposed that the progressively increasing hypocapnia leads to a progressively increasing potentiation of the reflexogenic signals thereby assuring an increasing dominance of the reflexogenic component.

Prolonged apneas resulting from maintained withdrawal of chemoceptive support during hypoöxic hyperpnea, frequently gave way to renewed breathing. This was attributed to reaccumulation within the center of acid derived from its own acid metabolism and to increasing acidemia.

Granting that a localized acidity of the chemoceptors is the stimulating influence producing a general alkalinization of the body during hypoöxia, the basic physiological chemical control of breathing (acid excess and O_2 deficiency) is again broadly interpretable in terms of the reaction theory. Not only is the activity of the center and of the chemoceptors explained but the changing relations of centrogenic and reflexogenic breathing during varying intensities of hypercapnia and oxygen deficiency are accounted for as well.

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THE EFFECTS OF EPINEPHRINE, POTASSIUM, PENTOBARBITAL AND INSULIN ON THE CONCENTRATION OF AMINO ACID NITROGEN IN THE BLOOD OF FASTING DOGS

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Many contradictory reports (1) concerning the effects of epinephrine on nitrogen metabolism have appeared; the most general finding is an increased nitrogen output following epinephrine administration. That this increase may be this result of increased deaminization is suggested by the findings of Luck and Morse (2) and Davis and Van Winkle (3), who report an hypoaminoacidemia after epinephrine administration in rabbits. It was desired, before studying the action of other agents which might themselves stimulate epinephrine secretion, to test this latter finding in the dog.

Because there have recently been a number of papers suggesting a relationship between the actions of potassium salts and epinephrine, most of them based on the circulatory effects of these two agents, we felt that a study of their possible similarity of action on the blood amino acid nitrogen level might afford an interesting biochemical check. On the one hand stands the contention of Camp and Higgins (6) that potassium is responsible for the effects of epinephrine, and that epinephrine functions only to regulate the distribution of potassium. The experimental basis of this contention, a similarity in the effects of the two agents on the circulatory system and smooth muscle, is confirmed by Mathison, McGuigan and Higgins (7) but denied by Hug (8). That epinephrine, in common with many other agents, does raise the serum potassium level by rapid mobilization of potassium from the lever is amply demonstrated (9), although the evidence offered by Larson and Brewer that the increase in arterial pressure caused by epinephrine is not dependent on a rise in the blood potassium level weakens considerably the postulate of Camp and Higgins.

On the other hand there is the statement of Hazard (10), supported by Katz and Katz (11) and by Hug (12), that potassium causes a secretion of epinephrine, which is in turn responsible for the observed effects of potassium. The reports of Brown and Feldberg (13) and of Feldberg and Guimarais (14) that potassium liberates acetylcholine from pre-ganglionic

fibers and is itself a direct stimulant to post-ganglionic fibers are compatible with this view, as are the findings of Hazard (10) and of Silvette and Britton (15) that potassium produces an hyperglycemia. The latter finding is opposed by Kylin (16), who reports a marked hypoglycemia after potassium as well as a weakening of the pressor action of epinephrine after potassium.

If either of these contentions is to stand in the face of biochemical evidence, it is necessary to discover whether the actions of epinephrine and potassium salts on the blood amino acid nitrogen level are similar.

EXPERIMENTAL. We attempted to investigate this by the use of healthy adult male mongrel dogs, weighing between ten and twenty kilograms, which were trained to lie quietly while blood samples of two and one-half cubic centimeters each were withdrawn from the saphenous vein, usually at hourly intervals during the experimental period. The dogs were fasted forty-five to forty-eight hours prior to the beginning of the experiment, and in general dogs were used in rotation so that each dog was employed only once a week. Determinations of amino acid nitrogen were made by the method of Danielson (17), modified only by the replacement of the colorimeter by a photelometer (Central Scientific Company) with a suitable filter (Cenco, no. 1, blue).

Fasting unanesthetized dogs. A series of fasting controls was run under the same experimental conditions as the other series in order to rule out the effects of fasting, hemorrhage (as reported by Bischoff and Long (20) and by Luck and Morse (2) as a cause of hypoaminoacidemia), and all manipulations involved in the experimental procedure, especially those which might cause apprehension and subsequent epinephrine secretion on the part of the dog.

Five experiments on four dogs constitute the series. A slight fall in amino acid level (0.7 ± 0.2 mgm. per cent) was noted, the significance of which when tested by the use of Fisher's "t" and probability tables (18) proved to be so small as not to appear on the tables.

Pentobarbital. A series of anesthetized control animals seemed desirable for two reasons: first, that an anesthetic would be necessary later during the administration of the highly irritant potassium; and second, that as many of the reports upon which contradictory conclusions are based mention the use of an anesthetic but do not rule out the possible action of the anesthetic, such a study might shed some light on the cause of disagreement.

Pentobarbital sodium was administered intravenously (in a dose of 27 mgm. per kgm. body weight) immediately after the pre-injection sample was withdrawn. Nine experiments were run, using five different dogs. The mean of the maximum falls in amino acid concentration after pentobarbital was 1.7 ± 0.3 mgm. per cent; when this is compared with the

corresponding value in the control series, the probability that such a difference could occur solely through errors of random sampling is about five in 100 (P = 0.0544): one can thus say with fair assurance that pentobarbital has caused a significantly greater lowering of the blood amino acid nitrogen than sampling or fasting alone.

Epinephrine. In the first epinephrine series, that on unanesthetized dogs and consisting of six experiments on five dogs, epinephrine hydrochloride was administered subcutaneously immediately after the pre-injection blood sample had been withdrawn. The dose used, 0.1 mgm. per kgm., always produced overt signs of epinephrine activity in the dogs. When the mean lowest post-epinephrine value is compared with the pre-injection mean, a lowering of 2.9 mgm. per cent is revealed, with a probability that this lowering is due to chance of less than two in 100 (P = 0.0194). When the mean of the maximum falls produced by epinephrine (2.9 ± 0.8 mgm. per cent) is compared with the corresponding value in the control series, we find again the chances that such a difference could occur by random sampling errors are only three in 100 (P = 0.0296). It is clear that epinephrine produces a marked lowering of the blood amino acid nitrogen level in unanesthetized dogs, a lowering significantly greater than that observed in the control series. This finding confirms the reports of previous workers and extends them to a different species, the dog.

In the second epinephrine series, introduced to afford a direct check on the possible mutual interaction of epinephrine and pentobarbital and to provide a more logical basis for comparison with the potassium studies, pentobarbital sodium, 27 mgm. per kgm. body weight, was administered intravenously to four dogs immediately after the pre-injection sample; a post-pentobarbital sample was drawn one hour later, and epinephrine hydrochloride, 0.1 mgm. per kgm. body weight, administered subcutaneously immediately after this sample. Again a slight lowering of the amino acid nitrogen level was observed. Comparison of the mean of the maximum falls in this series (1.8 ± 0.4 mgm. per cent) with like values in the three preceding series reveals that while this fall is significantly greater than that seen in the fasting control animals (P = 0.0442), it does not differ at all from that produced by pentobarbital alone (P = 0.6560), and is less in degree, though not significantly so, than that produced by epinephrine alone (P = 0.2554). It is interesting to compare this finding with that of Hrubetz and Blackberg (19) who report that pentobarbital impairs also the response of the blood sugar to epinephrine.

Potassium chloride. Here, as in the epinephrine group above, pentobarbital sodium, 27 mgm. per kgm., was administered intravenously immediately after the pre-injection sample. Post-pentobarbital samples were drawn hourly and potassium chloride 1.1 per cent (isotonic) was then run into the vein at such a rate that the animal received 1.5 mgm.

KCl per kgm. per minute for one hour, or a total of 90 mgm. per kgm.; samples were drawn when possible during the potassium infusion, immediately after cessation, and at hourly intervals thereafter. The series consisted of six experiments on four dogs.

A very slight fall in amino acid nitrogen was noted, the mean value at the end of the potassium infusion being 1 mgm. per cent lower and that one hour after the infusion 1.4 mgm. per cent lower than the mean preinjection value; in both cases the probability that such a difference might be equalled or exceeded through errors of random sampling is as high as one in four. When the average maximum drop (1.4 ± 0.4 mgm. per cent) is compared with that in the control series, the probability of such a difference as a result of random sampling errors is almost one in five. Potassium chloride did not, in the doses used and under the conditions of our experiments, cause a significant lowering of the blood amino acid nitrogen level of fasting anesthetized dogs.

TABLE 1

Summary of results

	MEAN LOWERING OF AMINO ACID CONCENTRATION	PER CENT OF PREINJECTION	"P" VALUE VERSUS CONTROL
	mgm. per cent		
Fasting control	0.7 ±0.2	9	
Pentobarbital	1.7 ±0.3	21	0.0554
Epinephrine	2.9 ±0.8	34	0.0296
Epinephrine-pentobarbital	1.8 ±0.4	24	0.0442
Potassium chloride-pentobarbital	1.4 ±0.4	17	0.1890
Insulin	2.5 ±0.3	30	0.0008

Because we felt that the failure of potassium in our experiments to cause a lowering of the blood amino acid nitrogen concentration might be the result simply of a sub-threshold dose, one acute experiment was run in which potassium chloride was injected to the point of cardiac arrest; no further change was noted in amino acid nitrogen concentration. We have thus failed to find biochemical evidence, using as our criterion the behavior of the blood amino acid level, that potassium is responsible for the effects of epinephrine or, on the other hand, that it causes marked epinephrine secretion, since we have shown that even a dose large enough to produce cardiac arrest does not significantly lower the concentration of blood amino acid nitrogen. Admittedly, some qualification of this statement is necessary, since the response to epinephrine was likewise impaired by pentobarbital. A final answer to the problem could be given if it were possible to administer potassium chloride to the unanesthetized animal, for in comparable experiments with epinephrine the results were unequivocal,—a

substantial fall in the amino acid nitrogen content of the blood was obtained invariably.

Insulin. In the course of further studies, not here reported, it seemed advisable to confirm previous reports (3, 4, 5) that insulin also produces a lowering in the concentration of amino acid nitrogen in the blood. The animals in this group, four in number, received insulin, 0.5 unit per kgm. body weight, immediately after the drawing of the pre-injection blood sample. The mean lowest post-insulin values were 2.5 mgm. per cent below the mean pre-injection values, and the statistical significance of this fall is unquestionable ($P = 0.0316$). If the mean of the maximum falls in this series (2.5 ± 0.3 mgm. per cent) be compared with that of the fasting controls, there remains no question that insulin produces a significantly greater lowering than fasting and sampling alone ($P = 0.0008$).

The results are summarized in table 1.

SUMMARY

1. The effects of epinephrine, pentobarbital sodium, potassium chloride, and insulin on the fasting blood amino acid nitrogen level of dogs have been studied.

2. It has been shown that both epinephrine and insulin produce a marked hypoaminoacidemia in unanesthetized fasting dogs.

3. Pentobarbital sodium, in anesthetic doses, has been shown to cause a significant though not a marked fall in blood amino acid nitrogen concentration.

4. However, when epinephrine was administered to dogs anesthetized with pentobarbital sodium, the resultant fall in blood amino acid nitrogen concentration was no greater than that produced by pentobarbital alone.

5. The intravenous infusion of potassium chloride, 1.5 mgm. per kgm. per minute for one hour, in dogs anesthetized with pentobarbital sodium, produced no significant effect on the level of blood amino acid nitrogen.

We are much indebted to Drs. F. L. Reichert and M. Mathes for their kind assistance in adrenodemedullation of one of the experimental animals. The results of this and of related experiments will be reported separately.

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DISTINCTION BETWEEN ARTERIAL, VENOUS AND FLOW COMPONENTS IN PHOTOELECTRIC PLETHYSMOGRAPHY IN MAN[1]

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This paper proposes to explore the possibility of distinguishing "active" from "passive" components, of separating arterial from venous reactions, in photoelectric plethysmograms of the human skin. Such distinctions appear possible through studies of the changes in blood content, in the volume pulse and in blood flow (calculated in arbitrary units) when these changes are recorded with photoelectric plethysmographs in the manner described below.

The possibility that these objectives might be served was suggested by several considerations. The excellent correlation found by Burton (1) between the amplitude of the mechanically recorded volume pulse and the blood flow in the finger indicated the desirability of studying more carefully the volume pulse in various skin areas in relation to the plethysmogram of the same area. Also, the use of the volume pulse as a measure of the abundance of the arterial blood supply in various skin areas (2) implied that it might serve as an indicator of arterial reactions in skin areas which can be profitably explored with the photoelectric plethysmograph. Variations in the amplitude of the volume pulse have received some attention as qualitative criteria of arterial reactions (1), (3), (5), but a more systematic exploration of this point seemed desirable from the viewpoint of the analysis of the vascular mechanisms involved in specific plethysmograms.

If the arterial reactions could be separated out from the plethysmogram with sufficient clarity, it seemed theoretically possible, under suitable circumstances, also to distinguish simultaneously the effects of arterial inflow, venous congestion and even of venous tone in the plethysmogram. The observations reported here show considerable promise in this direction.

METHODS. The experiments described below employ the photoelectric

[1] This investigation has been made with the assistance of a grant to A. B. H. from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

plethysmographs previously reported (2). When these are used in connection with resistance coupled amplifiers (4), the resulting records show the changes in blood volume and also in volume pulse amplitude. Careful inspection of the latter is most tedious. It is more practicable to record the volume pulses separately on a constant base line by employing a capacity-coupled amplifier. This permits higher amplification of the volume pulse and a correspondingly more legible record of it. This technique is equivalent to placing a leak sufficiently large in a mechanical plethysmograph to prevent changes in base line but not so large as to prevent adequate recording of the oscillations due to the pulse.

A convenient circuit for this purpose is shown in figure 1. Records taken with this amplifier and compared with those taken simultaneously

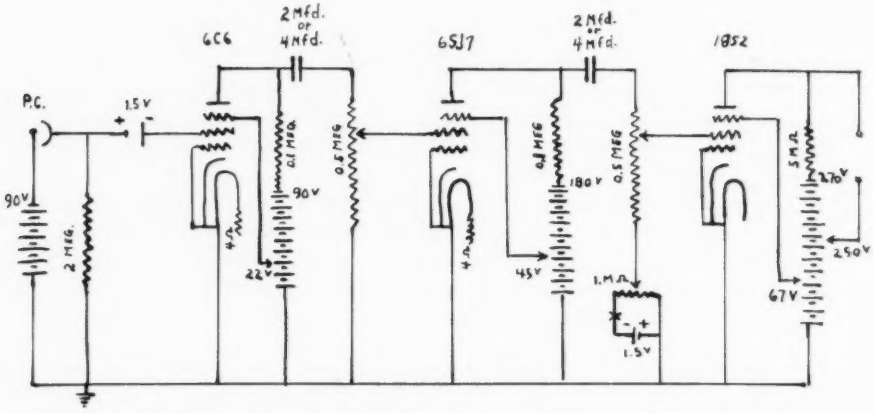

Fig. 1. Amplifier for recording the photoelectrically detected volume pulse on a constant base line. P. C.: photoelectric cell in plethysmograph. Tubes: 6C6, 6SJ7 and 1852.

from the same skin area with the resistance coupled amplifier (4), accurately show the amplitude of the volume pulse undistorted by changes in the position of the base line, when condensers of 2 mfd are used (fig. 2). Substituting condensers of 4 mfd provides for accurate recording of the wave form of the volume pulse but introduces minor variations in the base line which are of no importance in studying the wave form of the volume pulse, but which do interfere with following the variations in the amplitude of the wave. It should be noted that attempts to quantitate the volume pulse so recorded in terms of arbitrary but reproducible units (2) were not successful. True, calibration of the amplifier with standard input voltages is simple but irrelevant for the problem of the blood equivalent of the photoelectrically recorded volume pulse. Attempts to use the

"filter" technique for calibrating the volume pulse (2) ran into difficulties due to the discharge characteristics of the condensers and so were abandoned. This amplifier is not suitable for measuring the arterial blood supply of the skin.

One will observe that during constriction (fig. 2) the amplitude of the volume pulse decreases, the base of the recorded wave shifts upwards while the crest of the wave falls. This apparent shift in the base line is due to the fact that the pulse wave produces an oscillation either side of the mean which is the base line. When constriction occurs, the amplitude of the oscillation decreases in both directions toward the mean which lies nearer the base of the wave than its crest. As a result, the volume pulse so recorded mimics graphically the variations in artery diameter. This is extremely useful to the rapid inspection of arterial reactions.

Distinction of the arterial component in the plethysmogram. Simultaneous registration of the finger pad volume and of the finger pad volume pulse

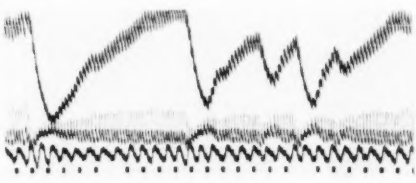

Fig. 2

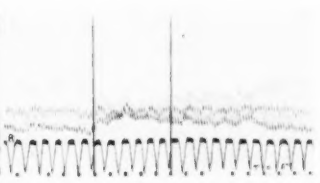

Fig. 3

Fig. 2. Finger pad plethysmogram (upper record), finger pad volume pulse. Respiration, time: 5 seconds. Spontaneous waves.

Fig. 3. Finger pad volume pulse. Respiration (re-traced). Time: 5 seconds. Normal subject. Inflation of arm-cuff at first signal, deflation at second signal.

as two separate records usually shows an excellent qualitative correlation when spontaneous waves (fig. 2), and the vasomotor responses to psychic stimuli, loud noises, a deep breath, and the cold pressor test are observed. The changes in volume are, in such responses, generally proportional to the changes in the amplitude of the volume pulse and they would therefore seem to be the result of altered flow due to the reactions of the controlling arteries. Hence, it is usually sufficient, and in practice more convenient, when desiring information only on the arterial reactions, to record the volume pulse alone.

An interesting application of this technique for separating out the arterial component in the plethysmogram is supplied in the case of a medical student who had developed a psychosis with respect to his own blood pressure. Every attempt to determine brachial arterial blood pressure on him with the cuff method elicited marked sympathetic excitement: increased heart rate, rise in blood pressure, sweating (particularly on the hands). Repeated physical examination of him for the purpose of com-

missioning in the medical reserve corps served to emphasize to him the fact that he was hypertensive. Since the normal subject responds with arterial constriction in the finger to inflating the arm cuff on the opposite arm (fig. 3), it seemed of interest to compare this man's reaction (fig. 4) with that of the normal subject. The negligible constriction in his fingers at the height of the reaction when his blood pressure reading was 160/80, contrasts strikingly with that occurring in the normal subject, suggesting that his hypertension was due to the cardiac acceleration. The contours of his arterial and digital pulse waves did not demonstrate arterial disease. We therefore felt that arterial disease was not the reason for the hypertension observed every time his blood pressure was measured. It is interesting that such disturbing sympathetic excitement may be present without much influence on the digital arteries since ordinarily they are exquisitely sensitive indicators of vasomotor reflexes and are probably the first ones to respond. The understandable reluctance of this subject to present himself for vascular studies which cause him real mental trauma

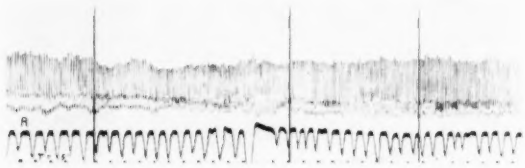

Fig. 4. Finger pad volume pulse. Respiration (re-traced). Time: 5 seconds. Subject has psychosis with respect to his blood pressure. Application of cuff to opposite arm at first signal, inflation at second, deflation at third signal. B. P.: 160/80.

has prevented further exploration of his case. It is therefore recorded here in its present unsatisfactory status.

There is usually a slight delay in the changes in volume with respect to the volume pulse (fig. 2). One gains the impression that this is related to the venous capacity of the pad and that the length of this delay is proportional to the venous capacity. The increase in transparency of the pad on pressing the blood out of the pad and preventing further inflow with a Gärtner capsule, varies considerably with different normal individuals. So also the decrease in transparency due to increased blood content of the pad on blocking the venous outflow shows marked differences in various normal subjects. The results with both of these procedures seem to correlate with the lag between the changes in volume pulse and volume. The difficulties inherent in securing quantitatively comparable measurements of the skin blood content with the photoelectric plethysmograph (2) have made further quantitative comparisons in this direction seem inadvisable at present.

The changes in volume with small spontaneous waves are relatively less than the changes in volume pulse at high rates of flow. This is reasonable since engorgement of the venous plexus by high flow would tend to maintain the volume constant despite oscillations in arterial tone which would exert their primary effect on the volume pulse.

Distinction of flow components in the plethysmogram. Although the blood flow through the finger is determined ordinarily by the finger artery tone, variation in the finger blood flow may result, independently of arterial tone, from changes in blood pressure and heart action. If artery tone is unchanged, the amplitude of the volume pulse would remain constant, while the blood content of the venous plexus would increase with an increased inflow due to increased heart rate. An occasional response in the finger pad to inhalation of amyl nitrite illustrates this relation (fig. 5). Finger pad volume increases for 35 seconds before greater am-

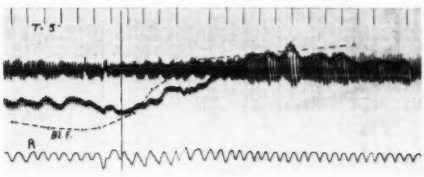

Fig. 5

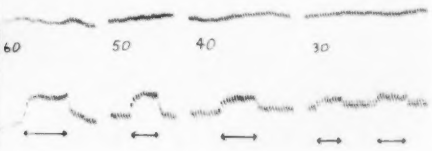

Fig. 6

Fig. 5. Finger pad volume pulse (upper record), finger pad plethysmogram (lower record). Time: 5 seconds. Respiration (re-traced). Bl. F.: Plot of product, volume pulse × heart rate, in arbitrary units. Amyl nitrite at vertical signal.

Fig. 6. Plethysmograms of neighboring finger pads. Gärtner capsule on proximal phalanx of finger giving lower plethysmogram. Pressures in capsule: 60, 50, 40, 30 mm. Hg in that order.

plitude of the volume pulse indicates onset of arterial relaxation. The rise in finger volume during this period tends to parallel the plot of the product of the heart rate and volume pulse amplitude, although there may be other components in this reaction as will be pointed out below. The maximum in the finger volume is reached when the value of this product is greatest. The value of the product, heart rate x volume pulse amplitude, seems to be a numerical expression in arbitrary units of the arterial flow. Although the validity of this relation awaits direct experimental verification with blood flow methods, the assumption of its correctness makes intelligible certain plethysmographic data such as are illustrated in figure 5.

It should be pointed out that failure in the correlation between changes in the volume pulse, in volume, and in flow, is observed only infrequently in the finger pad where variations in arterial tone appear to dominate blood flow and blood content. It is only under exceptional circumstances,

as illustrated in figure 5, that variations in flow may occur here independent of constancy in arterial tone with corresponding effects on volume. However, in the case of head skin where changes in arterial tone seem to be exceptional, the correlation between volume pulse, volume and flow is poor, due apparently to the operation of certain factors on the venous side of the circulation here.

Distinction of venous components in the plethysmogram. The very great physical difficulties involved in recording changes in venous tone have made us watch with especial care for any indication of varying venous tone in our plethysmograms. Theoretically, an increase in the blood content of the observed vascular bed independent of changes in arterial tone and in arterial inflow, or of mechanical factors on the venous side of the circu-

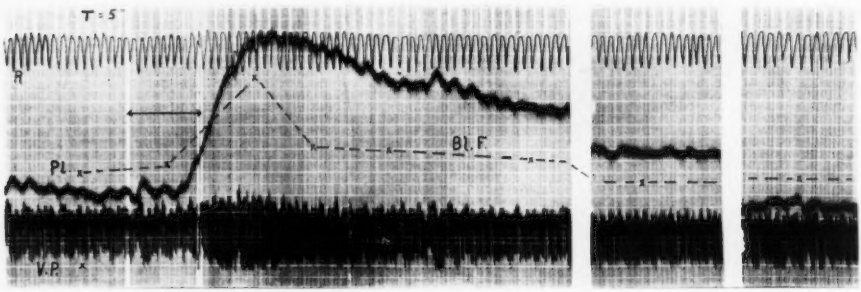

Fig. 7. Forehead plethysmogram and forehead volume pulse (lower record). Time: 5 seconds. Respiration (retraced). Bl. F.: Plot of product, volume pulse × heart rate, in arbitrary units. Ten minutes between first and second sections. Seven minutes between second and third sections. Amyl nitrite between signals.

lation, might be taken as evidence of dilatation of capillaries or veins or both.

The operation of mechanical factors such as obstruction to venous return and changes in intra-thoracic pressure, is usually evident in the plethysmogram and requires no illustration. It is interesting to note, however, that the blood content of the head skin varies much more readily with the intra-thoracic pressure than does that of the finger. Respiratory waves are more marked in the forehead plethysmogram. Effects on the venous drainage from swallowing, coughing, or a deep breath, are very obvious in the forehead, ear, and nose, although usually absent in the finger pad. The ease with which the venous blood content of head skin is altered is a complication in the study of arterial reactions in this area since the changes in volume may or may not be related to variations in arterial tone. We have been unable to decide whether these obvious differences in the behaviour of the venous drainages of the finger and

head skin are due to differences in the pressure in and extent and capacity of the venous plexus or to differences in venous tone. Engorgement of the small veins of the finger pad by obstructing venous outflow from the finger with a Gärtner capsule requires surprisingly high pressures. Values as high as 30 to 40 mm. Hg in the capsule are required in normal subjects to produce a detectable increase in blood content of the pad (fig. 6). This poses the interesting suggestion that these capsule pressures may measure the venous tone in the pad.

More direct indication of changes in venous or capillary tone is occasionally offered in the exceptional reactions to amyl nitrite inhalation (fig. 7). In this instance, arterial dilatation as indicated by the volume pulse occurred at the same time as the volume increased. The maxima are reached very nearly simultaneously in both curves, but recovery is strikingly different in the two curves. The volume pulse returned to the pre-administrational level in ninety seconds, but the volume required nineteen minutes for return. The plot of the product, volume pulse x heart rate, shows that this delay was not due simply to increased flow but argues for a temporary loss of tone on the venous side of the circulation here, thus accounting for the congestion. A similar loss in venous tone may also have been a factor in the interpretation of figure 5, but the parallelism between flow and volume is too close here to recognize a venous component in the reaction.

Discussion. The interpretation of these experiments is based on two relations for which the evidence is good but still incomplete:

1. *The amplitude of the volume pulse is a direct measure of arterial tone.* This relation seems to hold under normal circumstances (1), (2). It is a common experience in plethysmography that the volume pulse diminishes with arterial constriction as judged by direct inspection of the blood-vessels, by measurement of blood flow, and by such indirect criteria as temperature measurements. The accuracy of the relation is disturbed by changes in the position of the limb (6) but this source of error is not involved in the experiments reported here.

2. *The product, amplitude of volume pulse x heart rate, is proportional to blood flow.* This relation holds with considerable precision when the heart rate is constant, for then the product is directly proportional to the volume pulse which in turn shows good correlation with flow (1). The volume of the part then also correlates well with the amplitude of the volume pulse (fig. 2). The change in volume also correlates well with the product when both heart rate and volume pulse change and when there is no good reason to suspect venous congestion or change in venous tone. However, experiments are required for establishing the validity of this relation by direct measurement of blood flow. At present, the argument must rest on indirect evidence.

It follows from these two relations that the volume changes shown on the plethysmogram may be interpreted in terms of the individual contributions made by changes in arterial tone (under special circumstances). Thus, a plethysmogram showing increased volume and also increased volume pulse indicates arterial dilatation as a main factor in the increased flow and volume (fig. 2). If the calculated increase in flow parallels closely the increased amplitude of the volume pulse, arterial dilatation is the main component in altering the plethysmogram (fig. 2). If the calculated increase in flow parallels the product, volume pulse x heart rate, the volume pulse amplitude remaining constant, the increased flow is probably "passive" and does not involve arterial dilatation (fig. 5). Again, an increase in volume continuing after arterial dilatation and flow have returned to resting levels (fig. 7) may be due to venous congestion resulting from obstructed venous return or to loss of venous tone (fig. 7). Where there is no reason to assume obstruction to the venous return, one may infer a decrease in venous tone (fig. 7).

It is appreciated that gross changes in the dynamics of the heart beat will modify the validity of these theoretical considerations. It is appreciated that changes in the stroke of the heart may and do alter the amplitude of the volume pulse in a peripheral vascular bed such as the finger pad. But the effects of these factors are often recognizable. The cautious application of these considerations in photoelectric plethysmography in circumstances which probably do not influence heart action sufficiently to affect seriously the validity of these plethysmographic criteria, leads to useful information in the study of the circulation in the human skin.

SUMMARY

This paper studies the possibility of distinguishing "active" from "passive" components, of separating arterial from venous reactions in photoelectric plethysmograms of the human skin.

A technique is described for recording the volume pulse separate from the plethysmogram, with a photoelectric plethysmograph and capacity coupled amplifier.

The arterial component in the plethysmogram is distinguished by the amplitude of the volume pulse (figs. 2, 3, 4).

The flow component is indicated by the product, amplitude of the volume pulse x heart rate (figs. 5, 7). The value of this product appears to parallel flow.

The analysis of the volume changes recorded in the plethysmogram involves evaluating the arterial and flow components by these criteria and so by a process of exclusion, differentiating when possible the contribution of the venous component (fig. 7).

Measurement of the venous pressure in the finger pad by obstructing

the return with a Gärtner capsule indicates a surprisingly high venous pressure in the pad and also a high venous tone there (fig. 6).

Evidence is presented which seems to show that moderately heavy doses of amyl nitrite produce prolonged loss of venous tone (fig. 7).

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DO THE CAROTID SINUSES EXERT A PRESSOR ACTIVITY WHEN THE SYSTEMIC BLOOD PRESSURE IS LOW?

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In recent years much attention has been paid by many investigators to the part played by the nervous system in controlling the circulation under various conditions. As a consequence of this work discoveries have been made which are not only of physiological interest but which also shed light on a number of imperfectly understood clinical states. Outstanding within this field of research has been the work on the carotid sinus.

Hering (1) was the first to present real proof of the physiological activity of the carotid sinus. Hering (2) and later Koch (3) considered the sinus nerves as exercising solely a tonic inhibitory influence on the circulation. They believed the afferent discharge in the sinus nerves to be dependent on an adequate degree of distension of the sinus by the blood pressure. They regarded the rise of blood pressure and acceleration of heart rate on carotid occlusion as due to a decrease or abolition of sinus inhibitory action following the fall of endosinal pressure below the threshold value; they supposed that afferent impulses cease to pass up to the bulbar centers, which consequently overact. In support of this view is the fact that section of the sinus nerves or cocainization of the sinuses produces similar pressor effects.

Wright (4) does not believe it yet possible to exclude conclusively the existence in the walls of the sinuses of pressor fibers which are stimulated by the fall of pressure. Heymans and Bouckart (5) have found in perfusion experiments that when the pressure in the sinus of the dog was raised from 0 to 50 mm. of Hg, instead of getting no response as Koch (6) claims, they obtained a reflex rise in blood pressure. Wright (4) has found the same in the cat. McDowall (7) showed that after a hemorrhage, section of the vagi may produce a further fall of blood pressure, owing to the removal of the compensatory afferent pressor influence of these nerves. Stimulation of the central end of cut vagi causes an increase in the blood pressure, in some cases, which is indicative of the presence of pressor fibers. Using a technique similar to that employed by McDowall (7) I have studied

the carotid sinuses of dogs in an effort to determine the presence or absence of pressor activity in this structure.

METHOD. Large healthy dogs were used in this study. They were anesthetized with chloralose (60 to 85 mgm. per kgm. intravenously). Direct blood pressure from the femoral artery and respiration by the pneumographic method were recorded on the kymograph. The carotid sinuses were carefully dissected free and their activity determined. The results presented here are from only those animals (16 in number) which showed definite circulatory and respiratory reflex response to carotid occlusion and sinus massage. The vagi were severed to remove any compensatory

TABLE 1

DOG	WEIGHT	BEGINNING B.P.	PER CENT OF TOTAL BLOOD VOL. REMOVED	REMOVAL TIME	FINAL B.P.	EFFECT OF DENERVATION ON B.P.	
	kgm.	*mm. Hg*		*min.*	*mm. Hg*		*mm. Hg*
1	17	140	43	70	45	Rose gradually	15
2	20	120	50	65	45	Rose in next 5 min.	55
3	24	130	25	55	50	Rose gradually	20
4	18	145	40	55	45	Rose	15
5	20	130	31	80	60	Rose in next 5 min.	45
6	15	170	50	60	60	No change	
7	26	160	35	80	55	No change	
8	23	115	39	90	45	Rose in next 5 min.	25
9	22	130	30	65	50	Rose in next 10 min.	25
10	18	125	55	90	55	No change	
11	26	100	35	100	55	Rose in next 5 min.	30
12	20	110	35	60	55	No change	
13	18	140	60	80	60	Rose gradually	30
14	12	150	50	70	60	Rose in next few min.	40
15	18	145	40	50	70	No change	
16	25	115	30	90	70	No change	
Av.	20	133	40	72	55		30

activity by this route. The animals were bled slowly over periods varying from 50 to 100 minutes, from the other femoral artery. The bleeding was sufficient to lower the blood pressure to a sustained low level, previously selected, within the range of 45 to 70 mm. of Hg. It was felt that a pressure within this range would be sufficient stimulus to arouse pressor activity, if present. After making certain that the blood pressure was constant at the desired level the isolated carotid sinuses were rapidly denervated by mechanical stripping and painting with phenol. The latter was subsequently washed off with warm saline. All changes in blood

pressure, pulse rate or respiration in the next several minutes were read from the kymograph record.

Results. In ten of the sixteen animals there was a gradual rise of blood pressure varying from 15 to 55 with a mean of 30 mm. of Hg during the next few minutes following denervation. The rise in pressure in all cases was slow and gradual rather than abrupt. Six animals showed no change in blood pressure during a similar period and in no case was there a drop in blood pressure.

There were no appreciable changes in either respiration or pulse rate referable to the denervation.

Discussion. An increase in blood pressure, from a sustained low level, following denervation of the carotid sinuses indicates that functioning afferent depressor fibers were disrupted by this operation. This is contrary to Koch's (6) work for the dog, who found no depressor activity present in the sinus below about 60 mm. of Hg; neither does it substantiate the work of Heymans and Bouckart (5) who found pressor activity in the carotid sinus below 50 mm. of Hg for the dog, nor Wright's findings (4) of a similar nature for the cat. That compensatory measures could have been responsible for the rise in blood pressure in ten of the sixteen experiments does not seem probable, for the low pressures were maintained for 10 to 15 minutes previous to the denervation. Though the rise in pressure in those cases in which it rose was gradual, it was complete within one to five minutes in all but animal 9.

It is not surprising that there was no appreciable effect on pulse rate attributable to the denervation since the vagi were cut and Koch (3) has shown that the sinus reflex has relatively little effect on this factor as compared to the aortic reflex.

Respiration varied, as expected, to carotid occlusion, sinus massage and vagus section. At the low pressure most animals displayed a gasping type of respiration which in most cases was relieved at about 70 mm. of Hg in those animals whose blood pressure increased subsequent to sinus denervation. The change was not correlated with the denervation, but rather with systemic blood pressure, indicating that the gasping type of respiration was relieved by central effect rather than peripheral.

SUMMARY

Lowering of the blood pressure in dogs anesthetized with chloralose to sustained low levels varying from 45 to 70 mm. of Hg does not arouse any pressor activity in the carotid sinuses, which if present would be demonstrable by a fall in pressure when these areas were denervated. A gradual rise in pressure in 10 of the 16 experiments indicates the presence of depressor activity at the time of denervation. In the other 6 there was no change in blood pressure.

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RELATION OF THE GROUPS OF THE ADRENALIN MOLECULE TO ITS CARDIO-ACCELERATOR ACTION[1]

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Intrinsic differences in sympathetically innervated effectors were suggested by Elliott (1) to explain the excitatory effects of adrenalin on smooth muscle in certain organs and inhibitory effects on smooth muscle in other organs. With the demonstration of the adrenalin-like nature of the adrenergic mediator (2) the concept of differences in receptive mechanisms has been used to explain the effects of the mediator. The necessity for postulating two or more receptive mechanisms is evident, but the fundamental nature of these mechanisms is still unknown.

We have suggested that a possible difference in the reception of adrenalin by adrenergically innervated effector cells could be explained by assuming that certain groups of the adrenalin molecule are necessary for the effective union of adrenalin with the receptive mechanism in one type of effector, while one or more different groups unite with the receptive mechanism in another type of effector (3). It appears that this possibility may be tested by making quantitative comparisons of single-effector responses to adrenalin and to other phenylethylamine derivatives lacking one or another of the characteristic groups of the adrenalin molecule. Such a study has been completed with the denervated intestine in the unanesthetized dog (3). The following is a quantitative study of these compounds as accelerators of the denervated heart under experimental conditions identical to those used in the study of the intestine.

METHODS. At first dogs were prepared with denervated hearts by performing the series of three operations described by McIntyre (4). The right sympathetic trunk was removed from the stellate through the fifth thoracic ganglion, and the right vagus was cut below the recurrent laryngeal nerve. The corresponding part of the left sympathetic trunk was removed in a second operation. Following complete recovery from these two operations the left vagus was cut in the neck. After it was discovered that neosynephrin would produce a marked rise in blood pressure at an injection rate considerably below threshold for accelerating

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the rate of the denervated heart, this compound was used to test for the presence of cardio-inhibitory fibers. When cardiac slowing could be produced by neosynephrin in dogs having had the above three operations the presence of cardio-inhibitory fibers in the right vagus could be demonstrated by direct stimulation of the peripheral end of the cut nerve. The latter animals of the series were bilaterally vagotomized in the neck following recovery from bilateral sympathectomy in the thorax. These animals all experienced difficulty in swallowing and were regularly given glucose and saline intravenously. Records were obtained within one to three weeks after the vagotomy. None of the animals showed cardiac slowing during neosynephrin injection after sympathectomy and bilateral vagotomy in the neck. Four of the dogs had their adrenals demedullated by cautery previous to the cardiac denervation.

TABLE 1

Relative potency of adrenalin and simpler phenylethylamine derivatives as accelerators of the denervated dog heart

The reciprocal of each number in the table indicates the potency of the corresponding compound relative to adrenalin

		DOG								EXTREMES
		1	2	3	4	5	6	7	8	
Adrenalin (l)	$(OH)_2C_6H_3 \cdot CHOH \cdot CH_2NHCH_3$	1	1	1	1	1	1	1	1	1
Arterenol (dl)	$(OH)_2C_6H_3 \cdot CHOH \cdot CH_2NH_2$	1½	2	1½	2	1½	1½	1½	2	1 1½ ½ 2
Neosynephrin (l)	$m\text{-}(OH)C_6H_4 \cdot CHOH \cdot CH_2NHCH_3$				25	50	50	25-40	50	25 50
Epinine	$(OH)_2C_6H_3 \cdot CH_2 \cdot CH_2NHCH_3$				40	50	50	40-50	25-40	40 50
Synephrin (dl)	$p\text{-}(OH)C_6H_4 \cdot CHOH \cdot CH_2NHCH_3$				500-2500	500	500	1000-1500	1000	500 2500

Electrocardiographic records were taken from the denervated hearts under "near-basal" conditions during intravenous injection of the compounds from an electrically driven syringe. Details of the method have been described in a previous paper (3). The series of compounds used and the formula of each is shown in table 1. For each compound the lowest constant injection rate capable of causing a greater than five per cent acceleration of the denervated heart within less than 60 seconds was determined. Also each dog was given a series of adrenalin injections at rates from barely threshold to several times the threshold accelerator dose, with correspondingly greater acceleration, and these results were used as a scale for judging the accelerator potency of the other compounds injected at rates two to four times the threshold accelerator rate. The results below are obtained from analysis of 99 records from eight dogs.

RESULTS. *Minimal accelerator injection rate of adrenalin for denervated hearts of normal and adrenal demedullated dogs.* The minimal injection rate capable of causing more than 5 per cent acceleration of the denervated

heart in less than 60 seconds ranged from 0.00020 to 0.00057 mgm. per kilo per minute. Wada and Kanowoka (5) reported that injection of adrenalin at rates from 0.00020 to 0.00030 mgm. per kilo per minute was the minimum effective dose for accelerating the rate of the denervated heart and for increasing the blood pressure of non-anesthetized and non-fastened dogs having their adrenal glands demedullated. Dragstedt (6) has reported 0.00020 as the minimal pressor dose of adrenalin for un-anesthetized dogs. The sensitivity of the denervated Thiry fistula as an indicator for adrenalin in normal dogs is emphasized by these figures, since the denervated intestine is significantly inhibited by injection rates from 0.00010 to 0.00020 mgm. per kilo per minute.

TABLE 2

Acceleration of the denervated heart by adrenalin and simpler phenylethylamine derivatives

Dog 4, weight, 10 kilo, adrenal glands demedullated

	DILUTION	CUBIC CENTIMETERS INJECTED PER MIN.	HEART RATE PER MIN. FOR THE 15 SEC. BEFORE INJECTION	HEART RATE PER MINUTE FROM THE BEGINNING OF THE INJECTION CALCULATED FROM SUCCESSIVE 15 SEC. INTERVALS					
				1st	2nd	3rd	4th	5th	6th
1. Adrenalin	1:250,000	1	110	109	108	114	120	124	
2. Adrenalin	1:250,000	2	107	106	114	136	154	164	168
3. Adrenalin	1:250,000	2	112	114	118	133	140		160
4. Adrenalin	1:250,000	4	104	104	140	180	204		
5. Arterenol	1:250,000	2	117	116	123	132	134		
6. Arterenol	1:250,000	4	110	111	125	144	154	160	162
7. Neosynephrin	1:20,000	4	112	112	116	130	140	148	160
8. Epinine	1:25,000	1	108	107	107	106	106		
9. Epinine	1:25,000	4	104	104	104	106	110	114	
10. Synephrin	1:1,000	2	109	108	109	112	113	115	
11. Synephrin	1:100	2	107	106	116	142			

The minimal accelerator dose of adrenalin for the denervated heart was essentially the same for the animals with adrenals demedullated as for those with adrenals intact. Such a result would be explained if the basal output of adrenine from the adrenal glands is negligible, or if the denervated heart becomes more sensitive to adrenalin as a result of adrenal demedullation. Wada and Kanowoka (5) found that the basal rate of the denervated heart of the dog was unchanged by inactivation of the adrenal medullae.

Data from dog 4 shown in table 2 illustrate typical results obtained from continuous injection of adrenalin at low rates. An injection at the rate of 0.0004 mgm. per kilo per minute caused acceleration beginning in the third 15 second period. The rate increased steadily until 75 seconds

from the beginning of the injection when it was 14 beats per minute faster than normal. With an injection of 0.0008 mgm. per kilo per minute the acceleration began within the second 15 second period and increased almost linearly for two minutes until the rate was 50 beats per minute faster than normal.

When very low injection rates are sustained for several minutes, it is observed that acceleration of the denervated heart begins after a somewhat longer latent period than when larger doses are used, and the rate gradually increases until a level is reached which is maintained during an injection of 10 to 20 minutes' duration. For example, adrenalin was injected into dog 8 at a rate of 0.00012 mgm. per kilo per minute for 20 minutes. The normal rate was 122 beats per minute. During the 4th 15 second period after the beginning of the injection the rate per minute was 128; at the end of 3 minutes the rate was 142 and did not fluctuate significantly from this level until the end of the 20 minute period. This type of response suggests a building up of the concentration of adrenalin in blood for several minutes as the injection is continued and is difficult to explain otherwise. However, Rogoff and Marcus (7), using the "caval pocket" technique, detected no increase in the adrenalin content of the blood of the adrenal vein during continuous intravenous injection of adrenalin at rates many times as fast as those used in these experiments.

The ability of a near-threshold accelerator injection rate of adrenalin to maintain a fast rate of the denervated heart during the continued injection is to be contrasted with the failure of adrenalin to keep the intestine inhibited when injected at rates several times the threshold intestine-inhibiting dose (8). The denervated intestine becomes refractory to the inhibitory effects of adrenalin, while the denervated heart does not become refractory to the accelerator action of adrenalin. If, therefore, the cardiac rate and the intestinal motility be recorded simultaneously, an adrenalin injection rate may be used which results in both cardiac acceleration and intestinal inhibition at the beginning of the injection, while there is only cardiac acceleration at the end of the injection.

Relative potency of adrenalin and simpler phenylethylamine derivatives as accelerators of the denervated heart. Each of the four compounds, differing from adrenalin by lacking one or another of the three hydroxyl groups or the methyl group on the nitrogen atom, was capable of accelerating the denervated heart if injected in sufficient quantities. None of the compounds produced a slowing of the denervated heart. The difference in the potencies of the compounds as accelerators of the denervated heart is given in table 1. The extremes are listed for the increase in injection rates necessary to produce an acceleration comparable to that produced by a given dose of adrenalin. Therefore, the reciprocal of the number indicates the potency of the corresponding compound as compared with

adrenalin. A typical series of analyses used in determining relative potency of the compounds is shown in table 2.

The results for the denervated heart resemble those for the denervated intestine in that the greatest reduction in potency in each case is obtained by removal of the m—OH group of the adrenalin molecule, and the least reduction in potency results from removal of the—CH_3 group. In fact, since dl-arterenol (non-methylated adrenalin) was used in these studies, it can not be stated whether l-arterenol would be slightly more potent or slightly less potent than l-adrenalin. Removal of any one of the —OH groups of the adrenalin molecule results in a compound having much lower cardio-accelerator potency than adrenalin.

Since the results are concerned with compounds each lacking only one of the four groups which distinguish adrenalin from phenylethylamine, the question arises whether it may be concluded that all phenylethylamine derivatives simpler than adrenalin will be less potent. Such a generalization is not possible with regard to the pressor potency of phenylethylamine derivatives (9), but the pressor response is the result of the activity of various types of effectors and compensatory mechanisms. The generalization could not be expected to apply to anything but single-effector responses. Bacq's studies using the nictitating membrane (10) (11) indicate that tyramine, which lacks three of the groups investigated in this study, is less potent than any one of the three compounds lacking only one of these groups, and phenylethylamine is less potent than tyramine.

The minimal pressor injection rate for adrenalin in unanesthetized dogs is approximately the same as the minimal cardio-accelerator injection rate (see above). Removal of the para—OH group of the adrenalin molecule lowers the pressor potency to $\frac{1}{4}$ that of adrenalin but lowers the cardiac-accelerating potency to $\frac{1}{25}$ to $\frac{1}{50}$ that of adrenalin. Therefore, neosynephrin may be injected at a rate producing a pressor response without accelerating the denervated heart. Since adrenergic denervation in other tissues sensitizes them to neosynephrin (3), the innervated heart may be expected to be even less sensitive than the denervated heart to the direct accelerator action of neosynephrin. The only effect of neosynephrin on the rate of the innervated heart, with the low dosages, is reflex slowing as a result of the increase in blood pressure. When denervation of the heart was incomplete neosynephrin caused cardiac slowing, but in the completely denervated hearts no effect on rate was obtained. High dosages caused only acceleration of the denervated heart. Orth et al. (12) observed that neosynephrin was the only one of their series of sympathomimetic amines that did not markedly accelerate the sino-auricular rate under cyclopropane anesthesia. Also, neosynephrin was least apt to produce cardiac irregularities. When comparable pressor

dosages were used all of the compounds except neosynephrin increased the sino-auricular rate under ether anesthesia. The effects of this compound illustrate the fact that a general comparison may not always be made between the potency of adrenalin and other phenylethylamine derivatives. The comparison must be made with regard to a single type of effector. As judged by changes in potency in single effectors, removal of a single group of the adrenalin molecule may interfere to a greater degree with the reception of the compound in one type of effector than in another.

SUMMARY

The potency of adrenalin, arterenol, neosynephrin, epinine, and synephrin as accelerators of the denervated heart has been determined in normal and in adrenal demedullated unanesthetized dogs. Each of the latter four compounds differs from adrenalin by lacking one of the four groups that distinguish adrenalin from phenylethylamine.

The responses of the denervated hearts of the dogs with adrenals intact were indistinguishable from the responses of the adrenal demedullated dogs. This result with very low injection rates of adrenalin would be explained by a low resting output of adrenine from the adrenal medulla or, less likely, by a heightened sensitivity of the denervated heart to adrenalin after adrenal demedullation.

Development of refractoriness to the effects of a continuous injection of adrenalin and related compounds does not follow the same course in the sino-auricular node that it does in intestinal smooth muscle. Although the minimal effective doses are similar for the two indicators, continuous injection of adrenalin at low rates produces temporary intestinal inhibition and sustained cardiac acceleration.

Each of the compounds accelerates the denervated heart when given in concentration having any effect on the heart rate, and the degree of acceleration increases as the threshold accelerator dose is doubled or quadrupled. Removal of the —CH_3 group of the adrenalin molecule does not significantly affect the cardio-accelerator potency, but removal of any one of the three —OH groups results in a compound $\frac{1}{2^5}$ to $\frac{1}{2^{5^{6\,0}}}$ as potent as adrenalin. The most important of the four groups with regard to cardio-accelerator potency is the meta—OH group.

Removal of the para—OH group reduces the cardio-accelerator potency more than the reduction in pressor potency. Neosynephrin, given at a rate that will not alter the rate of the denervated heart, produces a rise in blood pressure. Neosynephrin, injected at this rate, serves as a test for cardiac denervation, since reflex slowing of the heart will occur if any cardiac nerves are present.

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THE EFFECT OF ADRENALIN ON CARBON DIOXIDE OUTPUT AND RESPIRATORY QUOTIENT: PROPORTIONALITY WITH DOSE

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This is the fifth and last of a series which has described the hyperglycemia (Griffith, Lockwood and Emery, 1939a), lactacidemia (ibid., b), oxygen consumption (Griffith, Emery and Lockwood, 1940a) and pulmonary ventilation (ibid., b) of chloralose-anesthetized cats during and following intravenous administration of adrenalin for 5-minute periods at carefully controlled rates from minimally effective to maximally tolerated.

The matter of final interest in connection with the metabolic effect of adrenalin is the mechanism of its calorigenic action. Attempted explanation of this has leaned heavily on the respiratory quotient and has consequently varied with the finding and interpretation relating to it, these having been about as various as its investigators (Erichson, 1936). The data to be presented here will show that dosage may be a predominant factor in this diversity; and when taken into proper consideration there is no evidence that adrenalin has an invariably specific effect on the respiratory quotient. Rather, the latter seems to reflect more closely than anything else the minute volume of pulmonary ventilation (1940b) and presumably, therefore, like it, is only unspecifically the resultant at any moment of at least blood pressure, metabolic rate and lactic acid acidosis.

PROCEDURE. These experiments being the same as those previously described, details of procedure need not be repeated. Suffice to say, respiratory exchange of these chloralose-anesthetized cats was determined by collection (for approximately 5-minute intervals) and analysis of expired air.

Usually (57 of the 84 experiments) two determinations of the "normal" resting respiratory exchange, 15 to 20 minutes apart, preceded injection. This, following immediately the second of these normals, or the single one when only one was made, was always for exactly 5 minutes and was accompanied by a collection of the expired air approximately coincident with it, thus affording information of the average, immediate effect for the 5-minute injection period. Two additional determinations were made during the subsequent half-hour recovery: one 5 to 10 and the other 25 to 30 minutes from the end of injection.

Parke-Davis adrenalin chloride was diluted with neutral, isotonic NaCl solution for injection (femoral vein) of 1 cc. per minute for 5 minutes so as to effect rates of administration in milligrams per kilo body weight per minute (number of experiments in parentheses) of: 0.00025 (10), 0.00050 (9), 0.00100 (9), 0.00200 (14), 0.00400 (13), 0.00700 (10), 0.01000 (9).

Control injections of isotonic, neutral NaCl (10 experiments) were similar in amount, rate and duration.

RESULTS. *Normal Values. Intra-individual stability.* Averages of the two normals, 15–20 minutes apart, preceding injection are: carbon dioxide output, 4.65 and 4.63 cc. per kilo per minute; respiratory quotient, 0.735 and 0.741. Thus, as confirmed also by the other functions measured in this work, the physiological condition of these animals at the time of injection was one of satisfactory stability.

Inter-individual variability. Carbon dioxide output, cubic centimeters per kilo per minute, varied in the 84 experiments from 2.7 to 7.7; mean, 4.81; standard deviation, 1.05; coefficient of variation, 21.9. This is not unlike the variability of the closely related oxygen consumption (Griffith, Emery and Lockwood, 1940a) and would seem, like it, not adventitious but an expression of the intrinsic metabolic variability of these animals; since correlation: with oxygen consumption is nearly one ($r = +0.948 \pm 0.014$); with pulmonary ventilation ($r = +0.781 \pm 0.027$), is of the same order of magnitude as the latter with oxygen consumption ($r = +0.729 \pm 0.038$); and with respiratory quotient ($r = +0.532 \pm 0.055$) is only as much greater than that of the latter and oxygen consumption ($r = +0.230 \pm 0.070$) as might normally be expected.

The average values for the various injection groups, cubic centimeters per kilo per minute (with rate of injection in parentheses) are: 5.4 (NaCl); 5.5 (0.00025); 4.6 (0.00050); 4.1 (0.00100); 4.0 (0.00200); 4.5 (0.00400); 6.1 (0.00700); 4.8 (0.01000). Injection at the lowest rate was ineffective (see below); and although the result following 0.007 mgm. per kilo per minute is slightly irregular it is not sufficiently so to affect the conclusions. The remaining average normal rates are sufficiently alike to show that extremes within each group were adequately balanced and to eliminate variable normal rate of putput as a possible determining factor in the effect of injection.

Respiratory quotient varied from 0.64 to 0.86; mean, 0.745; standard deviation, 0.048; coefficient of variation, 6.49. Twelve, or 14 per cent, of the determinations were below 0.70. In our experience this is not unlike the condition obtaining in normal, unanesthetized cats and is therefore probably not the effect of the anesthesia but characteristic of these animals in the post-absorptive condition. However, extremes within the

various injection groups (in parentheses) were adequately balanced to produce very similar average values: 0.73 (NaCl); 0.75 (0.00025); 0.73 (0.00050); 0.74 (0.00100); 0.75 (0.00200); 0.75 (0.00400); 0.76 (0.00700); 0.75 (0.01000); thus eliminating extremes of resting normal values as possibly affecting the average results to be described here.

The Effect of Control Injection of Isotonic NaCl. Averages of the 10 experiments in which isotonic NaCl was injected in amount equal to that

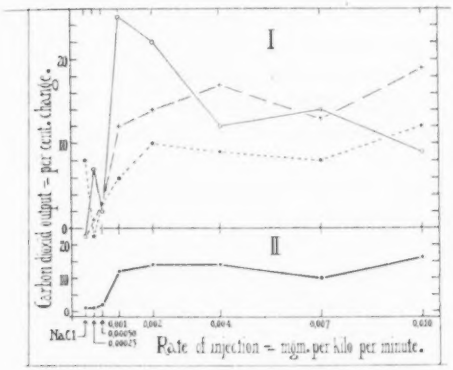

Fig 1

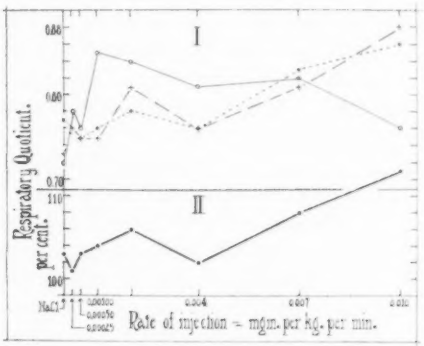

Fig. 2

Fig. 1. The effect upon the carbon dioxide output of chloralose-anesthetized cats of 5-minute intravenous injection of isotonic NaCl and of adrenalin administered at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute.

I. The upper set of three curves: *continuous line*, values in cubic centimeters per minute for the 5-minute injection period; *dashed line*, values for the interval 5 to 10 minutes after injection; *dotted line*, values 25 to 30 minutes after injection.

II. Total summated effect for the 35-minute experimental period, including the 5-minute injection and the 30 minutes following.

Fig. 2. The effect upon respiratory quotient of the injections of isotonic NaCl and of adrenalin described in the text and the legend of figure 1. The various curves have the same significance as there described.

The three curves of part I represent the respiratory quotient in actual values; the curve of part II represents the percentage change induced by each rate of injection.

serving as vehicle for the adrenalin in the remaining experiments showed percentage values for the 5-minute injection period, and 5 to 10, and 25 to 30 minutes after, of: carbon dioxide, 99, 99 and 108; respiratory quotient, 99, 100 and 105. The high terminal result is not typical and is due entirely to 2 aberrantly high values which are quite out of keeping with the small, evenly distributed plus and minus variations of the remaining 8 experiments. These, together with the equally negative results

of the 19 additional experiments employing the two lowest rates of adrenalin injection indicate nothing disturbing evaluation of a possible adrenalin effect is inherent in the experimental procedure itself.

These control values have been used as the initial points of the curves of figures 1 and 2.

The Effect of Adrenalin Injection. Figures 1 (carbon dioxide output) and 2 (respiratory quotient) present the results in two ways: part I of each figure includes three curves depicting the results during (continuous line), and 5 to 10 (dashed line), and 25 to 30 (dotted line) minutes after injection; the single curve of part II of each figure represents the approximate total change effected by each rate of injection for the entire 35-minute experimental period as obtained by summing the products of the values for the injection period and for the intervals 5 to 10 and 25 to 30 minutes after, when multiplied by 5, 10 and 20 (minutes), respectively; thus giving an approximate summation of the area beneath each of the individual curves relating the effect of each rate of injection to time.

Threshold. With both carbon dioxide output (fig. 1) and respiratory quotient (fig. 2) there is an apparently significant increase produced during the injection period (I, continuous line) by the lowest rate of administration, 0.00025 mgm. per kilo per minute. This, however, is not sustained at the next higher rate of injection (0.00050 mgm. per kilo per minute) and the total effect for the 35-minute experimental period is in both instances so nearly nil that no considerable error can result from considering the next rate of injection, 0.001 mgm. per kilo per minute, the really minimal effective dose.

This is exactly similar to the results previously described for both oxygen consumption and pulmonary ventilation; with both of these, also, the results of injection at the two lowest rates were ambiguous, the first unequivocal increase occurring likewise with injection at the rate of 0.001 mgm. per kilo per minute. And, finally, this was also the threshold rate of injection for increase of blood lactic acid, whereas hyperglycemia was definitely evident following injection at half this rate (0.00050 mgm. per kilo per minute).

Proportionality between rate of injection and increases of carbon dioxide output and respiratory quotient. Consideration of this relationship will be facilitated by taking up separately the changes effected: 1, during the 5-minute injection period; 2, 5 to 10 minutes, and 3, 25 to 30 minutes after injection; and 4, the total, summated effect for the entire 35-minute experimental period.

1. *Change in carbon dioxide output and respiratory quotient during the 5-minute injection* (figs. 1 and 2, parts I, continuous line): it will be seen that changes of both are so alike that description of one applies without qualification to the other; and, equally significant, both follow exactly the

same course as previously described for pulmonary ventilation (1940b). All three are maximally increased during the injection of 0.001 mgm. per kilo per minute; and, with minor irregularity probably of experimental origin and evident equally in each (i.e., of the results produced by 0.004 and 0.007 mgm. per kilo per minute, one is either slightly too low or the other too high) the effect is progressively less at each higher rate of injection.

Oxygen consumption during injection (previous report, 1940a) followed a totally different course; being increased approximately the same amount (between 6 and 8 per cent) by all rates of injection during this 5-minute injection-period itself. It therefore seems improbable that carbon dioxide output during this period of adrenalin action is of significance as a measure of the intensity or kind of metabolic process underlying the calorigenic effect of adrenalin.

Blood lactic acid was also affected quite differently (previous report, 1939b), increasing progressively (with minor fluctuations, again of probably experimental origin) from threshold to a maximum with the highest rate of injection. The exactly reciprocal variation of carbon dioxide output can in no way be taken, then, as directly related to the lactic-acid acidosis produced by adrenalin.

In the immediately previous report, also, it was shown that the changes of pulmonary ventilation during injection followed neither metabolic need as expressed by oxygen intake, nor acidosis as represented by blood lactic acid level; but were explainable only as an integrated response to these as modified by the inhibitory effect of a progressively increasing blood pressure. Since there is no means by which this latter could bear immediately upon carbon dioxide production, it must be concluded that in the exactly parallel variation of ventilation and carbon dioxide output it is the former which is determinative; i.e., carbon dioxide output in this phase of adrenalin action (during injection) has no metabolic significance, being to an indeterminate extent merely a passive result of varying degrees of "auspumpung" superimposed upon a probably steady increase in oxidative formation plus displacement by a lactic-acid acidosis.

Obviously (fig. 2) respiratory quotient could be substituted for carbon dioxide output in the above without change of meaning or interpretation. And since evidence on this point was one of the main objectives of the work which has been described in this series of reports it seems justifiable to point out that this conclusion, for which there has been some previous evidence (Erichson, 1926) appears hitherto never to have received such substantial proof as that afforded here.

Carbon dioxide output and respiratory quotient (2) 5 to 10, and (3) 25 to 30 minutes after injection (figs. 1 and 2, parts I, dashed and dotted lines, respectively): there is no need to consider the effects at these intervals after

injection in detail. In general, hypertension with its reciprocally inhibitory effect is eliminated as a decisive influence on pulmonary ventilation so that this (previous report) and, therefore, carbon dioxide output and respiratory quotient must be conditioned during this half-hour recovery period chiefly by metabolic rate and the lactic-acid acidosis.

Metabolic rate 5 to 10 and 25 to 30 minutes after injection, as measured by oxygen consumption, has been shown (1940a) to increase to a maximum following adrenalin administration at the rate of 0.004 mgm. per kilo per minute and thereafter to decline progressively after injection at the two highest rates; carbon dioxide output (fig. 1, dashed and dotted lines) during this period of recovery reflects, qualitatively, these variations of metabolic rate up to injection at the rate of 0.007 mgm. per kilo per minute. It is not to be supposed, however, that even within these rates of administration it is at these times quantitatively related to metabolic rate; displacement by the progressively increasing lactacidemia must contribute to the measured output and following the highest rate of injection becomes predominant; output being then increased in spite of the fact that oxygen consumption is not even appreciably affected (1940a).

Respiratory quotient (fig. 2, dashed and dotted lines) merely reflects these complicated relationships in a manner too intricate to justify or repay attempted analysis; particularly, its rapid elevation during this phase of recovery when injection has exceeded 0.004 mgm. per kilo per minute can have no metabolic significance.

4. *Total, summated changes of carbon dioxide output and respiratory quotient for the entire 35-minute experimental period* (figs. 1 and 2, parts II): it is possible that employment of sufficiently long experimental periods would permit compensation for the disturbances of carbon dioxide output and respiratory quotient that have been shown above to be characteristic of short-period determinations. This evidence indicates, however, that such compensation is not effected within half an hour after injection. Comparison of these curves with those previously given for blood lactic acid (1939b), oxygen consumption (1940a), and pulmonary ventilation (1940b) only emphasizes the conclusion already reached: that carbon dioxide output and, therefore, respiratory quotient following administration of adrenalin are merely unanalyzably complex resultants of the interaction of these three determinants; or, rather, follow more closely than any other the variations of pulmonary ventilation; which, in turn, is determined by the other two.

COMMENT. A study of the result of injecting adrenalin at any one of the effective rates employed in this work would reveal increased blood sugar and lactic acid concentrations, increased oxygen consumption, carbon dioxide output and respiratory quotient, and increased pulmonary ventilation; and the attempt to establish causal relationships between

any or all of these would have to depend on the degree of synchronism of the various changes. This has received considerable attention (for literature and pertinent data see Erichson, 1926) but for reasons not far to seek has been inconclusive. Thus as shown in this work, after injecting 0.004 mgm. per kilo per minute there is increase of all these variables during the 5-minute injection; this increase, for all except respiratory quotient continues at least into the 5–10 minute interval following and then all show parallel rate of decline toward normal. Following any other rate of injection, however, these relationships and correlations will be quite different so that conclusions based on such evidence will necessarily vary from one set of data to another depending on the effective concentration of adrenalin which is established by the method of administration employed. It was a suspicion of this which suggested that in so far as changes of any of these were of common or dissimilar origin the most crucial proof thereof would be their degree of alteration in response to varying concentrations of adrenalin, i.e., their proportionality to dose or stimulus.

It is this effect which has been described in this series of reports of which this is the last. These have shown that on this basis there is no exact correspondence between the calorigenic effect as measured by oxygen consumption and any of these variables. Perhaps this is not surprising in so far as changes of blood sugar and lactic acid are concerned; although if all three, as seems commonly supposed, are effects of uncomplicated direct cellular action it would not have been too surprising to find some similarity in their variation in response to a common stimulus. Unexpectedly, what little resemblance there is unites calorigenic response with mobilization of blood sugar rather than of lactic acid.

On the other hand it was hardly to be expected that calorigenic action, as measured by oxygen consumption, would show quite such complete independence of carbon dioxide output and respiratory quotient. These results more than substantiate such intimations of this as previously obtained and once for all render untenable any attempt to explain qualitatively the effect of adrenalin on metabolism from short-period measurements of the respiratory exchange.

SUMMARY

Data are presented describing carbon dioxide output and respiratory quotient during, and 5 to 10, and 25 to 30 minutes after, intravenous administration of adrenalin for 5 minutes at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute.

For the 5-minute injection period, maximum increase of both occurs with 0.001 mgm. per kilo per minute, which appears, also, the minimal effective dose. With each of the four higher injection rates, the increase

during this period of injection is progressively less. Such, also, was the effect on pulmonary ventilation, described in the previous report of this series (1940b); and thus is provided perhaps the most probable explanation of the results described here.

After injection, carbon dioxide output and respiratory quotient also seem most adquately explained by pulmonary ventilation, so, again, reference may be made to the description of it (1940b) for explanation.

Most importantly, neither carbon dioxide output nor respiratory quotient vary in proportion to dosage with any similarity to oxygen consumption and would therefore seem very unreliable indices of the qualitative metabolism underlying the calorigenic action of adrenalin.

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SOME FEATURES OF THE EARLY STAGES OF NEUROMUSCULAR TRANSMISSION

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In previous communications from this Laboratory (Rosenblueth and Morison, 1937; Rosenblueth and Luco, 1939) emphasis has been placed upon the complex sequence of variations of neuromuscular transmission when a motor nerve is stimulated at adequate rates. Three successive stages of transmission—indicated by initial high muscular tension, then low and thereafter again high tension—were recognized early during repetitive activation. These stages were followed by a further period of depression (the *4*th stage, fatigue) and by a later recovery (*5*th stage).

An occasional early stage of depression, different in its time course from the *2*nd stage, was mentioned by Rosenblueth and Luco (1939). The present study deals with this additional stage, the conditions for its regular appearance, its properties and its mechanism of production. At high-frequency stimulation this stage is later than the *2*nd. Indeed, it appears to take place during the course of the *3*rd stage (fig. 2). In order not to modify seriously the original nomenclature of the several stages the new phase will be spoken of as stage *3b*. The complete sequence of events includes, therefore, the stages *1*, *2*, *3a*, *3b*, *3c*, *4* and *5*; the three successive periods of depression, during which transmission fails to occur at many of the junctions, are *2*, *3b* and *4*.

METHOD. The muscle studied was mainly the soleus of the cat. Occasionally the gastrocnemius or quadriceps was observed, with only slight quantitative differences in the results. The cats were anesthetized with dial (Ciba, 0.75 cc. per kgm. intraperitoneally). The tendon of the muscle was freed and attached to a tension myograph. The muscles pulled against strong rubber bands. Upward excursions in the records denote contraction. The leg was fixed by drills inserted into the tibia or femur.

Stimulating shielded silver-wire electrodes were placed on the motor nerve, cut centrally. The stimuli were condenser discharges through a thyratron, controlled in rate by a frequency-beat oscillator. The intensity was maximal. The time constant of the discharges was approximately 0.2 msec.

The nerve action potentials were recorded sometimes from a pair of electrodes placed on an intact region of the nerve, peripheral to the stimulating electrodes. In some experiments the electric responses of the crushed peroneal nerve were led monophasically from the region near the head of fibula, the stimulating electrodes being, as usual, high in the thigh. The action potentials of the muscles were recorded diphasically from two silver or platinum needles inserted into the tendon and into muscle fibers, respectively. Capacity-coupled amplifiers and a cathode-ray oscillograph were used for the records.

Atropine and prostigmin (Roche) were injected intravenously. Acetylcholine and KCl solutions were injected into the central end of the tied inferior mesenteric artery; this insured a prompt delivery to the recording muscles and permitted the use of smaller amounts of the substances than necessary for intravenous injections.

RESULTS. A. *The stage 3b.* The existence of a secondary stage of depression and the clear temporal separation of this stage from the initial similarly depressed stage *2* were readily demonstrated by the two following experimental procedures. In some animals records were taken of the responses to a series of periods of repetitive stimulation with progressively higher or progressively lower frequencies. Figure 1 illustrates a typical instance. The popliteal nerve was stimulated for 15 sec. at the frequencies indicated, rest periods of 2 min. being allowed between successive stimulations. The existence of two separate stages of depression is clearly seen in the responses to 400 and 500 per sec. As will be shown later (fig. 4), the fall of tension which takes place at 200 and 300 per sec. is not due to fatigue, for if the stimulus is prolonged the tension rises within a few seconds or minutes.

The two depressions, *2* and *3b*, become even more prominent when a high frequency is applied repeatedly for short periods (10 to 15 sec.) with intervening similar short periods of rest. Figure 2 shows a typical record. It is apparent that the response to the first period of stimulation contained exclusively stages *1*, *2* and *3* (now designated *3a*). As the stimuli were repeated *2* became briefer and the bottom of its trough rose while *3b* started earlier and lasted progressively less time.

Repetition of stimuli leads to fatigue. The records in figure 2 may be explained on this basis. As fatigue progresses both *2* and *3b* become briefer, hence the increasing prematurity of their appearance; hence, also, the greater prominence of *3a* and *3c* (see p. 214). That repetition of stimuli within brief periods has also a favorable effect on the appearance of both *2* and *3b* is shown in figure 3. The experiment illustrated therein involved a combination of the two procedures reported above. The stimuli were repeated at regular short intervals and their frequency was first increased and then decreased. A comparison of the responses to 300 and

200 per sec. at the beginning and the end of the series (A and B, J and L-respectively) shows that the repeated stimulation has favored the occurrence of *2* and *3b* with relatively low frequencies. It may be inferred that the post-tetanic condition of the muscles is conducive to the appearance of the early depressions.

That the fall of tension which takes place early during stimulation at frequencies of 200 and 300 per sec. is not due to the development of

Fig. 1

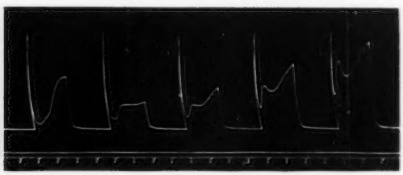

Fig. 2

Fig. 1. Appearance of stage *3b* and later of stage *2* as the frequency of stimulation is increased. Records from soleus. The popliteal nerve was stimulated for 15 sec. every 2 min. Frequencies of stimulation: A, 100; B, 200; C, 300; D, 400; and E, 500 per sec., respectively. Time signal: 5-sec. intervals.

Fig. 2. Effects of repetition of the stimuli upon the stages *2* and *3b*. Soleus. Frequency of indirect stimulation: 500 per sec. The record is continuous, the stimuli being applied repeatedly for 10 sec., with rest periods of 10 sec.

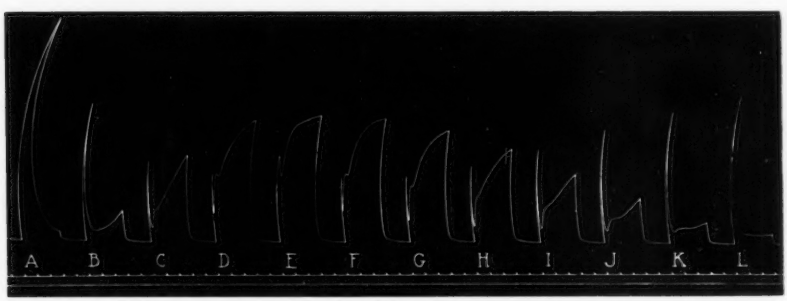

Fig. 3. Post-tetanic influence on the occurrence of *2* and *3b*. Soleus. Continuous record. Stimuli applied for 15 sec. with rest periods of 10 sec. Frequencies: A, 200; B, 300; C, 400; D, 500; E, 600; F, 500; G, 450; H, 400; I, 350; J, 300; K, 250; and L, 200 per sec.

the *4*th stage, but to the beginning of stage *3b*, is shown by the subsequent rise which occurs if the stimuli be prolonged over a period of 1 to 5 min. (fig. 4). With slower frequencies (e.g., 60 per sec.) the *4*th stage does follow the *1*st with no intervening *2* or *3* (Rosenblueth and Luco, 1939).

The occurrence of the several stages of neuromuscular transmission with different frequencies of stimulation may be summarized as follows. With low frequencies of indirect stimulation, e.g., 30 per sec. or less, transmis-

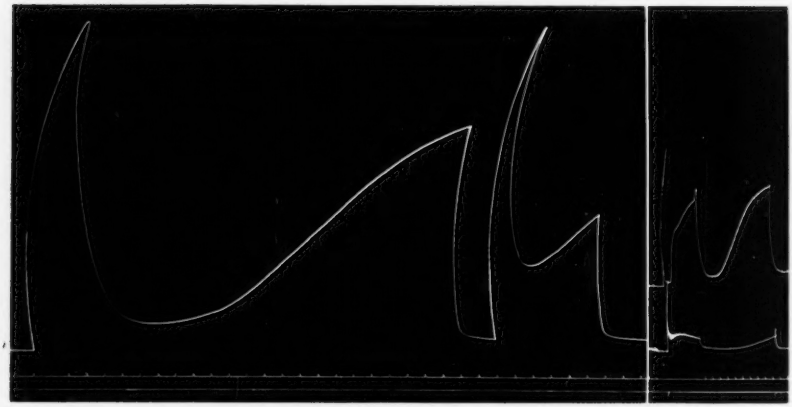

Fig. 4 Fig. 5

Fig. 4. Stage *3b* with a frequency of indirect stimulation of 200 per sec. Soleus. Time signal: 5 sec. After stimulation for 2 min. a rest period of 10 sec. was given. Re-application of the stimuli reveals the influence of fatigue.

Fig. 5. Stages *1*, *2*, *3a*, *3b* and *3c* elicited with a frequency of 30 per sec. after prostigmin. Atropine: 1 mgm. per kgm. Records from the two gastrocnemii. The left popliteal nerve (lower record) had been stimulated at 60 per sec. for 40 min. This stimulus was stopped and prostigmin (0.5 mgm.) was injected. Two min. later a frequency of 30 per sec. was applied to both sides (at the first lower signal). Time signal: 1 min. The drum was faster at the beginning of the response, to spread out the prompt and brief stage *2*.

TABLE 1

Changes in spike magnitude and in conduction velocity of the A fibers of a circulated peroneal nerve when stimulated for 15-sec. periods at different frequencies

The spike magnitude is expressed as per cent of the response of the rested nerve stimulated at a low frequency. The conduction velocity, expressed as m. per sec., refers to the fastest fibers in the nerve. The last four determinations at 500 per sec. were made successively with 10-sec. rest intervals between the periods of stimulation.

FREQUENCY PER SEC.	SPIKE MAGNITUDE					CONDUCTION VELOCITY			
	First spike	1 sec. later	5 sec.	10 sec.	15 sec.	1 sec.	5 sec.	10 sec.	15 sec.
100	100	105	103	105	100	97	100	100	98
200	100	110	105	105	105	100	98	97	97
300	100	107	103	103	100	97	95	95	93
400	100	104	103	99	97	93	90	90	89
500	100	103	99	99	95	90	88	85	82
600	100	110	103	101	100	83	78	75	74
1,000	100	86	62	43	41				
500	100	108	104	102	100	90	90	85	84
500	100	108	104	100	100	89	84	82	82
500	100	102	100	100		85	84	82	
500	100	108	102	100		87	82	80	

sion remains in the *1*st stage, even when the stimuli are applied for several hours (Rosenblueth and Luco, 1939). With frequencies between 50 and

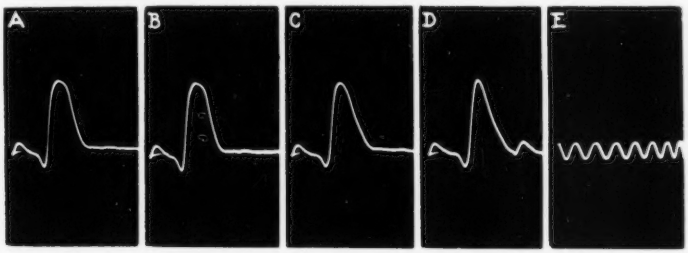

Fig. 6. Responses of the A fibers of a circulated peroneal nerve to high frequency stimulation. Sciatic cut centrally. Stimulating electrodes immediately below the cut. Recording electrodes near fibula; nerve crushed between them. Conduction distance 7 cm. The stimuli were made to trip the sweep of the cathode-ray oscillograph; the records begin, therefore, with a stimulus artifact.

A. Beginning of stimulation at 100 per sec. B. 15 sec. later. C. After 15 sec. stimulation at 300 per sec. D. After 15 sec. stimulation at 500 per sec. E. Calibration of sweep: 1,000 cycles.

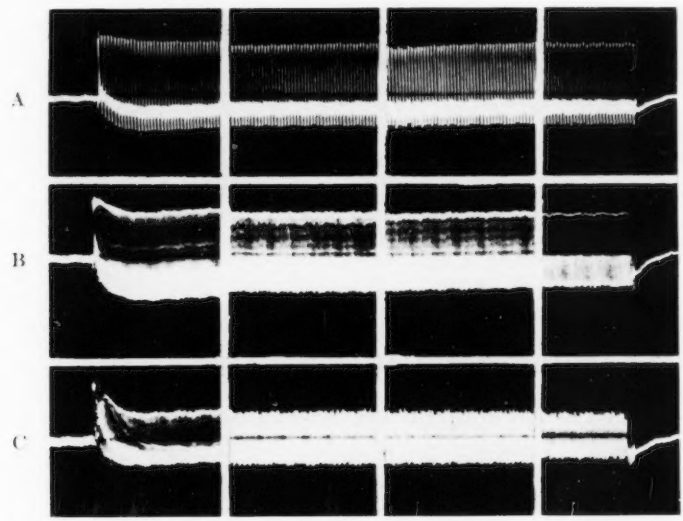

Fig. 7. As in figure 6, but continuous film to emphasize changes in spike-magnitude. The stimuli were applied for 15 sec. and pictures were taken at the beginning, 5 and 10 sec. later, and at the end of the period of stimulation.

A. Frequency, 400 per sec. B. Frequency, 800 per sec. C. Frequency, 1,000 per sec.

100 per sec. the *1*st stage is followed by the *4*th, and later by the *5*th. Between 150 and 300 per sec. the sequence is $1 \rightarrow 3b \rightarrow 3c \rightarrow 4 \rightarrow 5$.

With frequencies higher than 300 per sec. *1* is followed by *2*, then by *3a*, *3b*, and the sequence is thereafter as just given.

B. *The nerve action potentials.* Since there is little information available on the behavior of circulated mammalian nerves stimulated for relatively long periods with high frequencies, as was done in these experiments, the question arose whether the striking changes in tension illustrated above (figs. 1 to 4) could be due to corresponding changes in the number of nerve impulses delivered to the muscle per unit time. Accordingly the spike potentials of the A fibers of circulated peroneal nerves were recorded when stimulated as in the previous experiments.

Figures 6 and 7 illustrate typical results. For a more detailed description of the behavior of the nerves all the results of one experiment are sum-

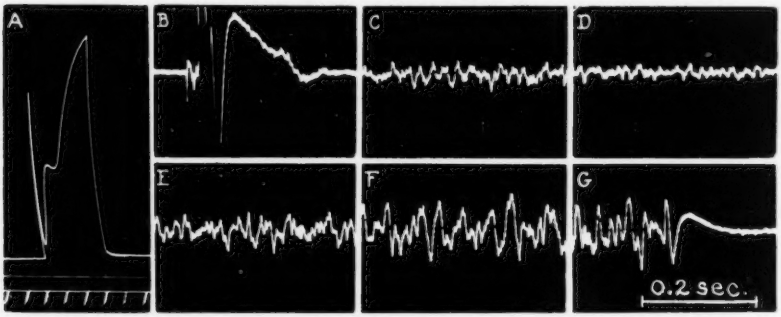

Fig. 8. Mechanical and electrical responses of a soleus muscle to high frequency (600 per sec.) stimulation of the popliteal nerve.

A. Mechanogram. Time signal, 5 sec. B to G. Electrograms. B, beginning of stimulation; the first spike potential went off the record. C to F at approximately 3-sec. intervals. G, end of stimulation.

marized numerically in table 1. With frequencies up to 600 per sec., although the conduction velocity is slowed, no significant change of spike magnitude of the α fibers takes place. Alternation was never apparent with frequencies up to 800 per sec., and was obvious at 1,000 per sec. only after a few seconds of stimulation. It is clear that the minor changes in the nerves are totally unrelated to the striking variations of neuromuscular transmission which correspond to the different stages.

C. *The muscle action potentials.* The electric responses of the muscles varied with the different frequencies of stimulation employed. It is not necessary for present purposes to give a detailed description of all the changes encountered. The following general statements may be made. At high frequencies of stimulation (300 or more) the muscle electrograms did not follow the rate of stimulation. Figure 8 is typical of the irregular

records obtained in all instances. Such irregularities are clearly indicative of asynchronism and alternation.

Notwithstanding the complex electromyograms recorded it was apparent (fig. 8) that the tension and the electric responses varied in a parallel manner. This parallelism warrants the conclusion that the changes of tension recorded indicate mainly or exclusively variations of contraction, not of contracture.

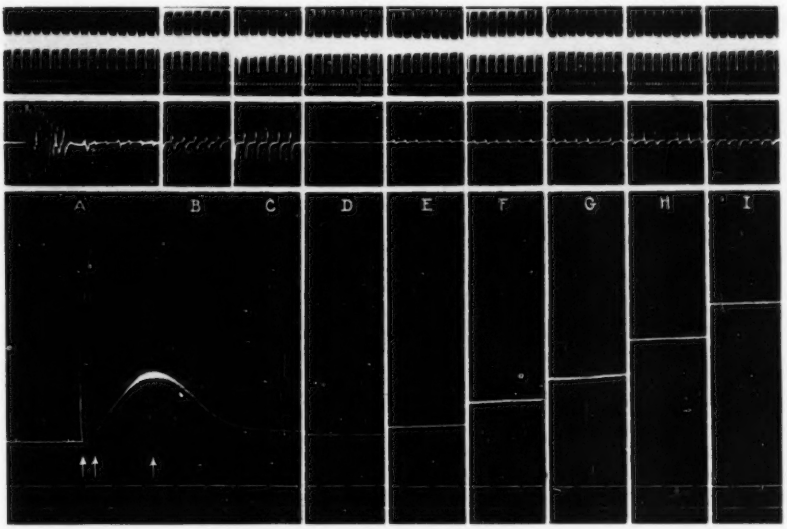

Fig. 9. Responses of nerve and muscle to a prolonged period of stimulation at 30 per sec. after prostigmin (0.5 mgm.). Upper records: nerve electrogram recorded diphasically from the stimulated popliteal nerve. Time: 10 msec. Middle records: muscle electrogram recorded diphasically from needles in the tendon and belly of gastrocnemius. Lower records: mechanogram. Time: 5 sec.

A, Beginning of stimulation. B and C, 3 and 12 sec. later, respectively (arrows). D to I, 2, 5, 6, 7, 10 and 14 min. later, respectively.

D. *Prostigmin.* Injections of prostigmin favor the appearance of stages *2* and *3b*—i.e., these stages occur with slower rates of stimulation than are necessary for their production in the normal systems. The results were most clear and striking when a slow frequency (30 to 60 per sec.) of stimulation was applied after atropine and prostigmin to a nerve which had not been stimulated previously. Figure 5 (upper record) illustrates a typical instance. Figure 9 shows that the nerve action potentials remain full-sized throughout the period of stimulation and that the changes of tension are due to increasing or decreasing numbers of muscle fibers sharing

in the response, as indicated by the variations in the muscle action potentials. When muscles had been stimulated for some minutes before the injections of prostigmin the stages in question did not appear as clearly as in fresh preparations (fig. 5, lower record).

When, after prostigmin, a high frequency of stimulation (100 per sec. or more) is applied to the motor nerve for a brief period (1 or 2 sec.)

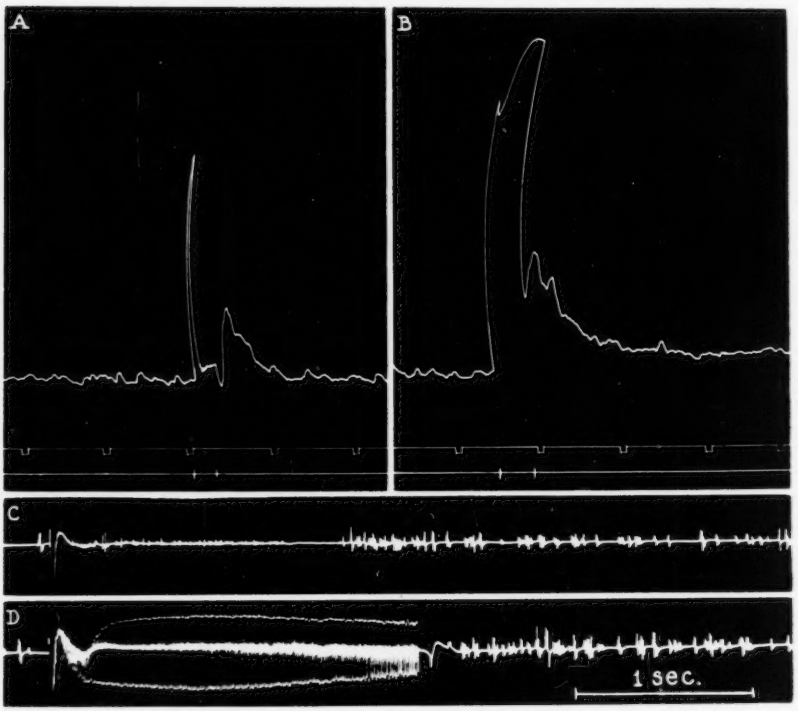

Fig. 10. Delayed contractions following repetitive stimulation after prostigmin. Soleus. Upper records: mechanograms; time signal: 5 sec. A, stimulation of popliteal nerve at 200 per sec. during period marked by lower signal. B, similar stimulation, but at 100 per sec. Lower records: muscle electrograms, C and D corresponding to the mechanograms A and B, respectively.

a delayed increase of tension takes place after stimulation has ceased (Rosenblueth and Morison, 1937). This phenomenon was frequently encountered in the present observations. The electromyograms (fig. 10) exhibit the presence of action potentials during this late response. The rise of tension is due, therefore, to a contraction, not a contracture.

E. *Acetylcholine.* Intra-arterial injections of acetylcholine were made

at different times during a period of stimulation at high frequency, in order to test the effects of these injections on the several stages of transmission. The results may be summarized as follows. As pointed out by Rosenblueth and Morison (1937), acetylcholine produces a fall of tension when injected during stages *1* or *3a*. This fall was then interpreted as the appearance of stage *2*. This interpretation is invalidated by the present observations. Acetylcholine had only a slight and inconsistent effect on stage *2* (fig. 11A). On the other hand, it caused a marked accentuation and prolongation of stage *3b* (fig. 11A and B). The fall of tension which

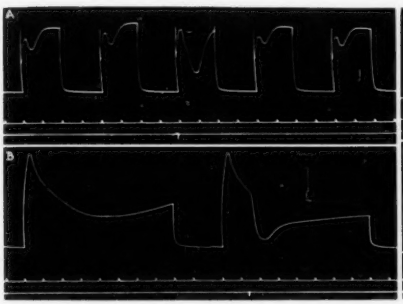

Fig. 11

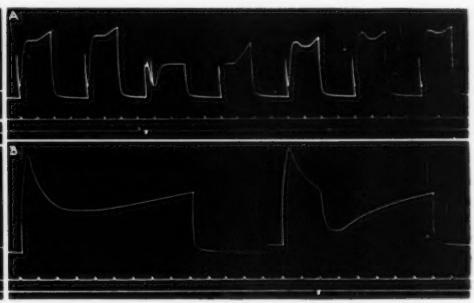

Fig. 12

Fig. 11. Influence of acetylcholine on stages *2* and *3b*.

A. Plantaris. The popliteal nerve was stimulated at the rate of 600 per sec. for 10 sec. with 10-sec. rest intervals. Several such stimuli had been applied before the record began, so that a steady state of the responses had been attained. At lower signal (i.e., at the start of a period of stimulation) acetylcholine (1 mgm.) was injected into the inferior mesenteric artery. Time signal: 5 sec.

B. Soleus. The first response shows the result of stimulation at 200 per sec. A similar response is markedly depressed by the injection of 0.5 mgm. acetylcholine at the lower signal. Time: 5 sec.

Fig. 12. Influence of potassium on stages *2* and *3b*. Records and procedure as in figure 11, except that KCl (20 mgm. in A, 25 mgm. in B) was injected instead of acetylcholine.

acetylcholine produces when delivered during stage *1* is therefore probably due to the development of stage *3b*.

F. *Potassium.* The results of intra-arterial injections of KCl were qualitatively similar to those of acetylcholine described above. Small doses (5 to 15 mgm.) had a brief action, while larger doses (20 to 50 mgm.) had prolonged effects. Figure 12 illustrates typical observations.

G. *Post-tetanic effects.* It is well known that the responses of muscles to different stimulating agents and procedures undergo characteristic variations when tested shortly after a period of tetanization. In the course of this study several such changes were encountered, which will be described in this section.

Rosenblueth and Morison (1937) reported that the muscular responses to single nerve volleys could become repetitive during the post-tetanic period. This report was called erroneous by Brown and Euler (1938). Feng, Li and Ting (1939a), however, gave clear electrical evidence of the repetitive nature of the post-tetanic responses of soleus. We were able to confirm fully these observations.

The responses of denervated muscles to acetylcholine were found by Rosenblueth and Luco (1937) increased after a period of direct tetanization. In the present experiments the responses to both acetylcholine and potassium were greater after short periods (10 to 30 sec.) of indirect activation. Indeed, doses which were quite subliminal before the tetanus yielded marked effects during the post-tetanic stage. Such responses were con-

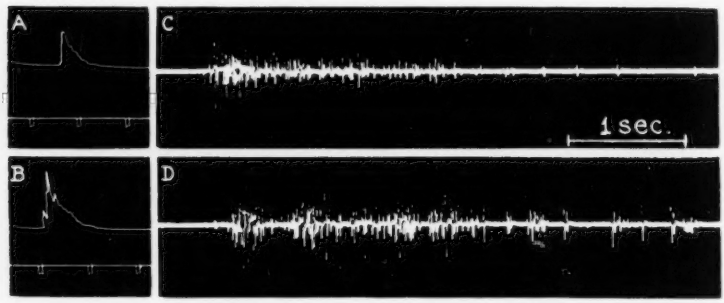

Fig. 13. Mechanical (A and B) and electrical (C and D) responses of a soleus muscle to intra-arterial injections of acetylcholine (1 mgm., A and C) and KCl (50 mgm., B and D). These injections were made shortly after several periods of tetanic stimulation. The same doses injected before tetanic stimulation gave no responses, mechanical or electrical.

tractions, as shown by the presence of spike-potentials in the electrograms (fig. 13).

Discussion. Changes of tension early during repetitive indirect stimulation at high frequency had been observed by Wedensky (1886) and Hofmann (1902). A recognition that such changes of tension imply different stages of neuromuscular transmission was, however, not made at that time.

The records in figures 2 and 4 indicate that stages *2* and *3b* are not phenomena of fatigue or exhaustion. If such were the case repetition of the periods of stimulation at short intervals should lead to increasing fatigue, and hence to a deeper and more prolonged fall of tension at those stages. The opposite effect is observed: stages *2* and *3b* become briefer and less prominent as fatigue develops. The paradoxical increase of response dur-

ing fatigue is explained by the assumption that stages *2* and *3b* are not caused by deficiency or exhaustion, but are due to the superabundance of a depressive agent or condition.

Repetition of the periods of stimulation does result, in certain observations, in an increase of stages *2* and *3b* (figs. 2 and 3). It may be inferred that a previous period of tetanization conditions the succeeding response in two opposite ways. It tends to decrease the response (both the + and the − stages) because of fatigue. But it also tends to increase these responses because of the development of another factor, unknown at present. This factor is assumed to be responsible for the changes described in section G under the heading "post-tetanic effects." It will be discussed further below.

Before considering the possible mechanisms involved in the appearance of the several stages it is important to emphasize some of the negative aspects of the problem. The records of the nerve spike-potentials (figs. 6, 7 and 9, table 1) render unlikely the view that changes in the magnitude of the nerve impulses delivered to the muscle can be responsible for the sequence of the stages. It is true that these spike-potentials are measured from the axons in the nerve trunks, not at the fine terminal filaments or nerve endings. But there is no reason to believe that such fine nerve terminals differ significantly in their properties from the regions of the axons tested. Indeed, the sequence of the stages takes place after prostigmin at such low frequencies (figs. 5 and 9) as to render quite improbable the view that the nerve impulses would vary significantly during the period of stimulation.

The electromyograms (figs. 8 and 9) indicate that the changes of tension are due to variations of contraction, not to the presence or absence of contractures. Although systematic data on the ability of the conducting system of muscles to follow different frequencies are not available, the incidental observations made in this and previous studies would eliminate changes of muscular conduction as the source of the sequence of the stages. At slow frequencies (fig. 9) the muscle should be capable of following the stimuli throughout and although at high frequencies the muscle cannot follow the rate applied to the nerve, typical action potentials are elicited at a slower rate (fig. 8).

It may be inferred that the sequence of stages denotes fundamentally changes of neuromuscular transmission, as opposed to possible changes in the conducting systems of either nerve or muscle.

As far as is known at present, there is no significant difference in the process of transmission during stages *1*, *3a* and *3c*. The stimulus delivered by the nerve to the muscle is adequate. There is no alternation in the muscular responses at low frequencies, and, if the rate of the nerve impulses

is too high for the muscle to follow, alternation is grossly similar in these three stages. The action of acetylcholine, potassium and prostigmin is likewise similar.

The stages of depression or lack of transmission, *2*, *3b* and *4*, on the other hand, differ significantly from each other, as follows. With respect to frequency of stimulation, stage *4* occurs at lower frequencies than the other two; a frequency of 50 per sec. is adequate for its appearance in gastrocnemius or soleus (Rosenblueth and Luco, 1939). Stage *3b* takes place with frequencies of 200 to 300 per sec. (figs. 1, 3 and 4), while stage *2* requires frequencies of 400 or more (figs. 1, 2 and 3). Acetylcholine and potassium have only a slight effect on stage *2* (figs. 11 and 12); they markedly accentuate stage *3b* (i.e., the tension falls lower; figs. 11 and 12); they improve transmission during stage *4* (i.e., the tension rises; Rosenblueth and Morison, 1937).

When a single early stage of depression is present in the response to stimulation under given experimental conditions, it is sometimes difficult to decide whether that stage is *2* or *3b*. If the action of acetylcholine or potassium can be tested the doubt may disappear. It is likely, however, that in certain conditions—e.g., rapid stimulation after prostigmin—stage *2* is long-lasting and stage *3b* begins sooner than usual, so that the two stages merge and stage *3a* is totally absent. Such a sequence can only be inferred.

Rosenblueth and Morison (1937) attributed stage *2* to an excess of acetylcholine causing the well-known paralytic action. This view is not supported by the present data. For, if stage *2* were due to an excess of acetylcholine, injections of the substance should result in further depression, yet they do not (fig. 11). On the other hand, stage *3b* appears to be produced by precisely that mechanism (fig. 11). The excess of acetylcholine could be absolute, because of accumulation during repetitive stimulation, or only relative, because the muscle presents to the transmitter a lower paralytic threshold at that time. That high frequency of stimulation will render the muscle more excitable to acetylcholine is shown in figure 13. This increased excitability after high-frequency activation is in contrast with the decreased excitability which is apparent after low-frequency tetanization (Rosenblueth, Lissák and Lanari, 1939). The complexity of the after-effects of tetanic stimulation is thus emphasized.

If stage *2* is not due to an excess of acetylcholine, what may be its source? This question is at present unanswerable. Clearly some depressive factor different from acetylcholine is at play. Indeed, since acetylcholine, which deeply modifies the responses of the muscle at all other stages, has a minor effect during stage *2*, it is necessary to assume further that the development of this factor is inversely related to the concentration of acetylcholine. Boyd, Brosnan and Maaske (1938) have described an early inhibitory stage

apparent in neuromuscular systems treated with magnesium. Although the data are not sufficient for a decisive conclusion, it is possible that this inhibition may differ from stage *2*, since the authors conclude that their depression is more marked at lower than at higher rates of stimulation, whereas *2* is in direct relation with the frequency of stimulation (figs. 1 and 3).

Feng, Li and Ting (1939a) report that the repetitive response of a muscle to a single nerve volley during the post-tetanic period is followed by an inhibitory after-effect. This inhibition is decreased by eserine. It is furthermore independent of the frequency of stimulation (Feng, Li and Ting, 1939b), the first volley producing as much after-depression as a series of volleys. Since stage *2* is more prominent after eserine and since it is undoubtedly a function of the frequency of stimulation, it may be inferred that stage *2* and Feng, Li and Ting's inhibition are due to different processes.

It may be concluded that the processes which may lead to cessation of neuromuscular transmission are numerous and complex. The explanation suggested by Rosenblueth and Morison (1937) for stage *4*, that it is due to an exhaustion of the production of acetylcholine, fits all the data (cf. Rosenblueth, Lissák and Lanari, 1939). Stage *3b* may well be due to an excess of acetylcholine; no data contradict this view. At least two more conditions of depression should be recognized: stage *2* and Feng, Li and Ting's inhibition. More data are necessary before deciding upon a reasonable explanation for these two types of depression which will be more than speculative.

The increment of responses to single nerve volleys during the post-tetanic period was attributed by Rosenblueth and Morison (1937) to a mobilization of potassium ions in the muscle. A similar explanation has been adopted by Brown and Euler (1938) and by Feng, Lee, Meng and Wang (1938). In accord with this view Lee (1939) reports that there is in denervated muscle an inverse correlation between the potassium contents and the excitability to acetylcholine. Before applying Lee's evidence to the problem under discussion it would be necessary, however, to learn whether the responses of the muscles he tested were contractions or contractures.

The data (figs. 11, 12 and 13) support the close interrelation between acetylcholine and potassium in neuromuscular transmission previously suggested by many observations. The nature of this interrelation is, however, at present quite obscure. It is likely that light on this problem might elucidate many of the difficulties now encountered.

SUMMARY

The responses of striated muscles to stimulation of their motor nerves were studied in cats. With a high frequency of stimulation the tension undergoes the following typical sequence of ups and downs. The initial

rise (stage *1*) is promptly followed by a fall (stage *2*); a further rise (*3a*) is again followed by another fall (*3b*); a new rise (*3c*) marks the end of the "early" stages; these are followed by the "late" stages, fatigue (*4*) and a delayed rise (*5*).

When tests are made with increasing frequency of stimulation stage *3b* occurs with a lower rate than is necessary for the appearance of stage *2* (figs. 1, 3 and 4). Repetition of a high frequency results in the earlier development of the stages of depression *2* and *3b* (figs. 2 and 3).

The depressions are not due to corresponding decreases in the nerve impulses delivered to the muscles (figs. 6, 7 and 9; table 1).

The changes in tension are due to variations of contraction, not of contracture (figs. 8 and 9).

After prostigmin the early stages may occur with relatively slow frequencies (figs. 5 and 9). A brief period of stimulation at a rapid rate is usually followed by a delayed contraction (fig. 10).

Acetylcholine and potassium have only a slight effect on stage *2*. They accentuate the depression during stage *3b* (figs. 11 and 12).

Tetanic stimulation augments the muscular responses to acetylcholine and potassium (fig. 13).

The discussion of the data leads to the following inferences. The changes of tension are due to presence or absence of transmission at some of the neuromuscular junctions—i.e., the stages denote changes of transmission (p. 215). No explanation is available for stage *2* (p. 216). Stage *3b* can be explained by the assumption of a paralytic effect of an excessive concentration of acetylcholine (p. 216). The increased responsivity of muscle to nerve impulses, to acetylcholine, and to potassium after a period of tetanization may be due to a mobilization of potassium during the tetanus, but direct evidence is lacking of such mobilization and its mechanism (p. 217).

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SOME CONDITIONS AFFECTING THE LATE STAGES OF NEUROMUSCULAR TRANSMISSION

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When a motor nerve is continuously stimulated at the rate, e.g., of 60 shocks per second the muscular response becomes minimal as time passes. This stage of "fatigue" is followed gradually by a remarkable recovery of the ability of the muscle to contract (Luco and Rosenblueth, 1939). Because of earlier variations in the behavior of muscle stimulated indirectly these two stages have been numbered, respectively, *4* and *5*. In an assay of the acetylcholine (a-ch) content of motor nerves which had been stimulated until the fatigue of stage *4* or the full recovery of stage *5* had been reached, Rosenblueth, Lissák and Lanari (1939) found that the the concentration of a-ch was low in stage *4* and that it gradually increased with the degree of recovery in stage *5*. According to the theory of chemical transmission at neuromuscular junctions, stage *4* results from *transmission fatigue*, i.e., the output of a-ch is not adequate to excite contraction in many of the muscle fibers; and in stage *5* the muscular performance is better because the nerves, richer in a-ch, are liberating more of it at their terminals. Support for this interpretation of stages *4* and *5*, or lack of it, is important for acceptance or rejection of the chemical theory. Further pertinent evidence can be obtained by testing the effects of prostigmin and curare and by registering the influence of tetanic stimulation in the two stages.

METHODS. The method employed was essentially that described by Luco and Rosenblueth (1939). In cats under dial anesthesia the Achilles tendons, which were attached to two similar levers, pulled downward against heavy rubber bands. Usually all three muscles—gastrocnemius-plantaris-soleus—were left attached to the tendon, but occasionally the soleus was used alone. For rigid support the legs were fixed by drills driven into the tibiae and firmly clamped to reinforced uprights. Shielded electrodes applied to the popliteal nerves, after severance of the sciatic as near to its origin as convenient, carried the condenser discharges (regulated by a thyratron) which served for stimulation. Shocks of such intensity as to produce maximal single contractions were invariably used.

RESULTS. *The action of prostigmin in stages 4 and 5.* Because pro-

stigmin protects a-ch from rapid destruction by cholinesterase it might be expected to improve muscular contraction in stage *4* by favoring greater effectiveness of the meager output of a-ch from the nerve terminals at that time. In stage *5* it might have either favorable or unfavorable effects, dependent on whether the preservation of a-ch increased the concentration of that agent strictly within the stimulatory range or raised the concentration above the paralytic threshold. Furthermore, since stage *5* develops concomitantly with an increase of a-ch in the nerve, prostigmin might be expected to affect the rate of its development.

In preparation for the tests the animals were secured against the harmful action of prostigmin by an intravenous injection of atropine (1 mgm. per kgm.) just before stimulation was started. The stimuli were repeated at the rate of 60 per second. The popliteal on one side was stimulated until stage *5* was first evident, whereupon stimulation was started on the other side. When on this second side stage *4* was approached or actually reached, prostigmin was injected into the jugular vein. Figure 1 is an illustrative instance of the difference of the effects of a small dose on the two responses — mainly a depressive action in stage *5* and a purely augmentive action when stage *4* was about to be entered. It is noteworthy that the further the progress into a fully developed stage *5* the more direct is the depression induced by prostigmin (cf. fig. 1). And when stage *5* is more marked on one side than on the other, as indicated by a greater degree of tetanic contraction, the effect of prostigmin in causing relaxation is greater on the more contracted side.

Tests with single shocks revealed that the potentiative action of a small dose of prostigmin (about 0.1 mgm. per kgm.) will last at least 90 minutes; and other tests, e.g., on the appearance of stages *2* and *3b* (cf. Rosenblueth and Cannon, 1940), indicated that its influence may persist for even longer periods. It was reasonable, therefore, to look for an effect of prostigmin on the time required for the first sign of stage *5* after the stimulation started, and also on the rate of development of stage *5* after it was started. The procedure used to secure evidence on these points was to stimulate the popliteal on one side until stage *5* began to be manifest, and then inject prostigmin (usually 0.5 mgm.) and initiate stimulation of the other popliteal. In six experiments stage *5* invariably developed sooner under the influence of prostigmin than in its absence. The period was shorter by intervals varying from 15 to 60 minutes (see table 1). Usually the time required for the first appearance of stage *5* after the start of stimulation (at 60 shocks per sec.) lies between 90 and 120 minutes. Not only does prostigmin advance the beginning of stage *5*, it also accelerates its development. For example, the degree of shortening of the muscles in stage *5* that had been reached just before the first injection of prostigmin at 12:08 in figure 1 had required 43 minutes from the start of that stage.

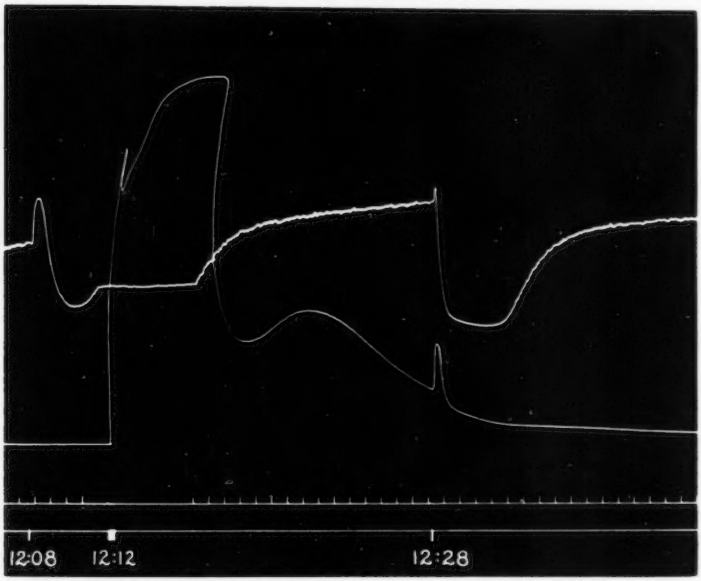

Fig. 1. The effects of prostigmin in stage *5* (upper record) and near the beginning of stage *4* (lower record). At 12:08 prostigmin (0.25 mgm.) was injected into the jugular vein with no obvious effect on the resting muscle (lower record before stimulation). After 12:12 the drum was turned rapidly in order to bring out stage *2* when the stimulation of the resting side was started; stages *3a*, *3b* and *3c* follow. At 12:28 prostigmin (0.25 mgm.) caused a pure contraction on the side approaching stage *4*, and on the side in stage *5* almost a pure relaxation. Time, 1-minute intervals.

TABLE 1

Time of first appearance of stage 5 after the start of stimulation.

In each experiment are compared bilaterally symmetrical muscles in the same animal.

WITHOUT PROSTIGMIN	AFTER PROSTIGMIN	SHORTENING OF THE PERIOD
min.	*min.*	*min.*
85	65	20
105	45	60
118	65	53
75	60	15
105	75	30
135	120	15

After the two injections of prostigmin (0.25 mgm. each) the same degree of shortening in stage *5* was reached by the muscles of the other side in 22 minutes. This more rapid increase of tension in the recovery phase after

fatigue, as the stimulation is continued under prostigmin, is a quite typical phenomenon.

The relative effects of curare in stages 4 and 5 and at the fresh neuromuscular junction. Curare reduces the muscular response to uniform maximal nerve impulses or to uniform injections of a-ch (Brown, Dale and Feldberg, 1936; Rosenblueth and Luco, 1937). By increasing the amount injected the response is decreased. In short, curare acts as if it raised the threshold to a-ch. Since there is evidence that nerve impulses discharge different amounts of a-ch in stages 4 and 5 and when there has been no previous

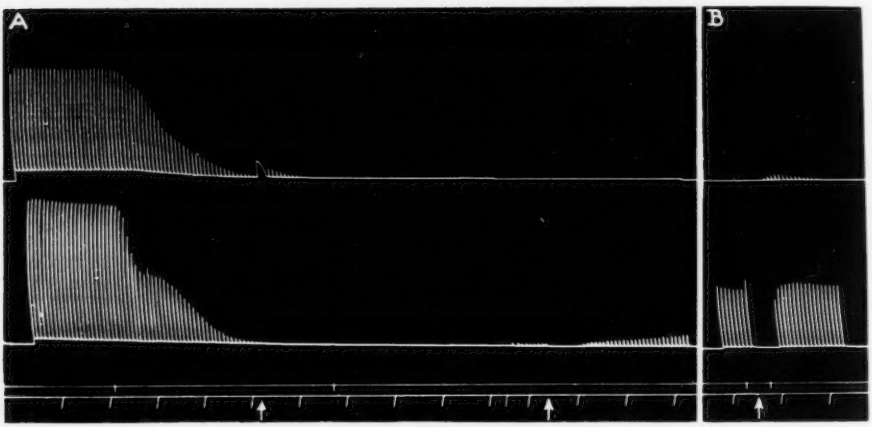

Fig. 2. Records of two soleus muscles, simultaneously stimulated through the popliteal nerves with maximal single shocks every 5 seconds. Upper record, nerve-muscle fresh; lower record, in stage 4.

A. At signal, 0.15 cc. of curare injected intravenously. Time, 1-minute intervals. Three and 9 minutes after the injection (at first and second arrows) the nerves were stimulated 10 and 30 seconds, respectively, at the rate of 60 shocks per second.

B. Twenty minutes after the injection. No response on the fresh side until after tetanic stimulation (30 sec., 60 shocks per sec., at arrow). Good responses of the soleus in stage 4, both before and after temporary inhibition during the rapidly repeated shocks.

stimulation, curare might be expected to have different effects in these various circumstances.

First, two symmetrical muscles (soleus or gastrocnemius), one in the fresh state and the other in the fatigued condition of stage 4, were compared while being stimulated at 5-second intervals with maximal single shocks applied to the popliteal nerve. The intravenous injection of a small amount of curare then showed that the response of the fatigued muscle is depressed sooner than is the response of the fresh one. Furthermore, this earlier effect is followed by a sharper decline in the height of the contractions. These results are shown in figure 2.

Although fatigue at the neuromuscular junction is attended by a prompt indication of the depressive influence of curare, the recovery from curarization begins sooner and is accomplished more rapidly on the fatigued side than on the fresh side. In the experiment illustrated in figure 2, for example, the first slight signs of recovery appeared in the lower record (stage *4*) about 9 minutes after the injection; on the other hand, except as post-tetanic contractions (fig. 2B), they did not appear in the upper record (fresh)

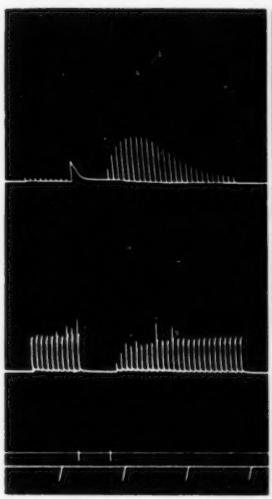

Fig. 3

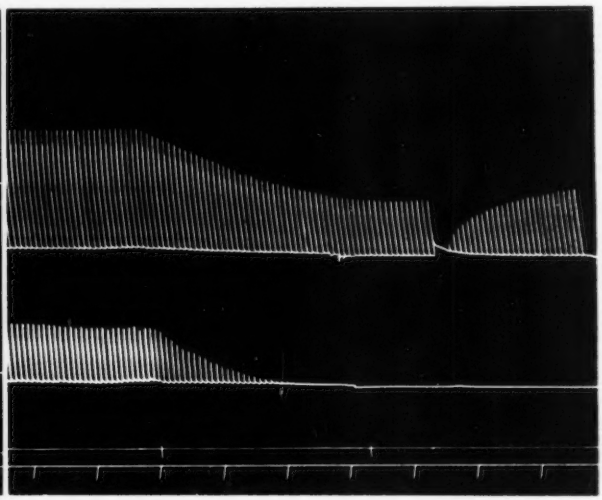

Fig. 4

Fig. 3. Records of two gastrocnemius muscles simultaneously stimulated through the popliteal nerves with maximal single shocks every 5 seconds. Upper record, nerve-muscle fresh; lower record, in stage *4*. Curare injected 12 minutes previously. Between signals, tetanic stimulation (60 per sec.) for 30 seconds produced Wedensky inhibition. A post-tetanic decurarization occurred on the fresh side only.

Fig. 4. Records of two gastrocnemius muscles stimulated simultaneously through the popliteal nerves with maximal single shocks every 5 seconds. Upper record, in stage *4*; lower record, in stage *5*. At first signal curare (0.05 cc.) injected intravenously; at second signal artificial respiration started. Time, 1-minute intervals. The interruption in the upper record, about 4 minutes after the injection, was due to 10 seconds of stimulation (of both nerves) at 60 shocks per second.

until 31 minutes after the injection. It seems, therefore, that although curare acts earlier at the fatigued neuromuscular synapse, it has there a less profound and less persistent effect, i.e., the fatigued synapse is more resistant than the fresh one (cf. fig. 3).

Another remarkable difference between the performance of a fresh neuromuscular preparation and one in stage *4* is seen in testing for post-tetanic decurarization. A short tetanic stimulation, at high frequency,

applied to a nerve in a curarized animal, will cause temporary decurarization at the nerve terminals (Boyd, 1932). This typical phenomenon does not occur in stage *4*. As shown in figure 3 the gastrocnemius in stage *4* (lower record) had partially recovered from curare, while the other gastrocnemius (fairly fresh) was only slightly responsive to the test nerve volleys (5-second intervals). Then a tetanic stimulation, at 60 per second, was applied. Continuance of the single shocks disclosed a striking post-tetanic decurarization on the fresh side, but none on the other side, in stage *4*.

Still another difference between the fairly fresh neuromuscular synapse and the synapse in stage *4* is disclosed when, after curarization has been partially recovered from, the nerves are maximally stimulated at a slow rate (2 per sec.). The decline in the degree of contraction as the stimuli are repeated (Wedensky inhibition) is much more marked and more rapid in the fresh than in the fatigued muscle. No definite conclusion can be drawn from this contrast, however, because the rate of recovery from curarization is different on the two sides (see p. 223). The contrast is mentioned here as a matter of record.

A comparison of the action of curare on stages *4* and *5* shows that it is much more depressant on the latter than on the former, i.e., the resistance to curare, increased during stage *4*, decreases towards the normal as stage *5* develops. An intravenous dose (0.15 cc.) which does not stop respiration may completely stop the responses to maximal single shocks (delivered at 5-second intervals *via* the popliteal) of a gastrocnemius in stage *5*, while merely reducing to about one-half, for a few minutes, the responses of the other gastrocnemius in stage *4*, tested in the same manner (see fig. 4). This is a typical phenomenon.

It is worth noting that Wedensky inhibition, produced by slowly repeated stimuli (2 per sec.) after partial recovery from curare, is more prominent in stage *5* than in stage *4*. Here again the 5th stage resembles the fresh condition. Comment on the relations of stage *5* to stage *4*, however, is made difficult in the circumstances by the differential recovery from curarization in the two conditions.

The post-tetanic increment of contractions in stages 4 and 5. When a series of twitches of skeletal muscle, evoked indirectly by stimuli of uniform intensity, is interrupted by a brief tetanus, the subsequent twitches are greater than the original. Clear evidence is lacking to account for this well-established phenomenon. Further information concerning the conditions which affect its appearance is therefore important.

As shown in figure 5A, a typical augmentation of the single responses occurred after 15 seconds of stimulation of the soleus through the popliteal nerve at the rate of 500 shocks per second. Thereupon the muscle was similarly stimulated for 42 minutes, at the rate of 60 shocks per second,

until stage *4* was well established. Almost immediately after cessation of the fatiguing stimuli, maximal single shocks at 5-second intervals were begun, and at the end of a minute tetanic stimulation (500 per sec.) for 15 seconds was repeated. When the single shocks were continued, the post-tetanic increment failed to appear (see fig. 5B). After a short rest period—4 or 5 minutes—the augmenting influence of the tetanus was again manifest, but not to so great a degree as in figure 5A.

When stage *5* had been developed by further stimulation at 60 shocks per second, the absence of the increment was still more striking (see fig. 5C).

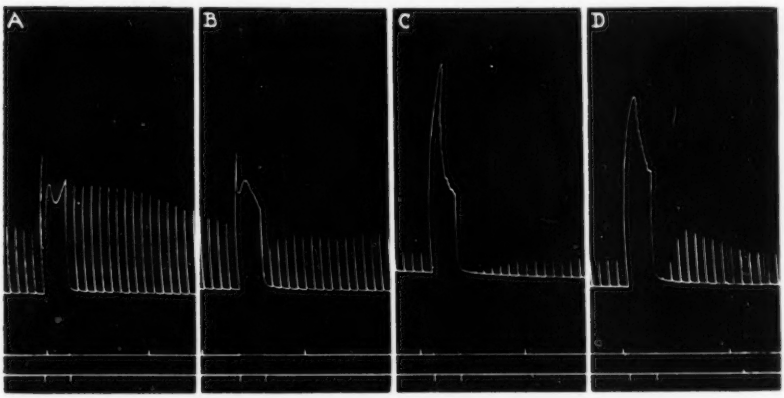

Fig. 5. Records of the right soleus muscle stimulated through the popliteal nerve. Time, 1-minute intervals.

A. Increment of responses to maximal single shocks (repeated at 5-sec. intervals) after 15 seconds of tetanic stimulation at the rate of 500 shocks per second.

B. Absence of the post-tetanic increment when the same test was applied, but after stage *4* had been produced by stimulation of the muscle 60 times per second for 42 minutes.

C. Absence of the post-tetanic increment immediately after interruption of stage *5*.

D. Reappearance of the increment, somewhat belated, after 10 minutes of relative freedom from stimulation.

Three tests during 12 minutes after the end of the stimulation which produced stage *5* elicited no post-tetanic increase of response, in spite of two rest periods of 3 minutes each. Ten minutes later, however, a rather belated increase was recorded (see fig. 5D).

Incidentally it may be reported here that our observations have confirmed those of Feng *et al.* (1938) that the soleus muscle is better suited to manifest the post-tetanic increment than is the gastrocnemius, and that the longer the conditioning tetanic stimulation, within limits, the longer the duration of the subsequent increment.

Discussion. *The effects of prostigmin.* As pointed out earlier, there is indication of a low output of a-ch in stage *4* and of an increased output in stage *5*. The results obtained by use of prostigmin are consistent with that evidence. Prostigmin augments the muscular response in stage *4*, and has chiefly a depressive influence on the response in stage *5* (see fig. 1). In both stages the drug protects a-ch against rapid destruction by cholinesterase. The greater resultant contraction in stage *4* can reasonably be interpreted as due to the participation of a larger number of fibers, brought into action because the persistence of a-ch results in an increased concentration at the synapse. The diminished response in stage *5* is reasonably explained by the development of so high a concentration of the protected a-ch that it reaches up into the paralytic range for many of the fibers (cf. Rosenblueth and Morison, 1937).

Likewise can be explained the influence of prostigmin in shortening the period between the start of stimulation and the first sign of stage *5*, as well as shortening the period of development of stage *5* itself. Stage *5* grows out of stage *4* as a-ch becomes gradually more concentrated in the nerve fibers. By checking destruction of the slowly increasing amount of a-ch in the nerve when the period of fatigue has begun to pass away, prostigmin naturally sets forward the process of recovery.

Rosenblueth and Luco (1939) have shown that there is no correlation between the height of the spike potentials of the stimulated nerve and the development of stage *5* in the responding muscle and thereby they have proved the inadequacy of the electrical theory in explaining stage *5*. That theory also fails to account for the action of prostigmin. Even if prostigmin should have a sensitizing effect which would render electrical impulses from the nerve fibers more effective in stage *4*, that effect could not be reconciled with the depressant action of prostigmin in stage *5*. On the other hand, the theory of chemical transmission, as shown above, can readily elucidate these phenomena.

The effects of curare. Difficulties are encountered in attempting a complete explanation of the quantitative differences in the action of curare at the various stages of neuromuscular transmission. The more prompt depression of response of muscles in stage *4*, as compared with fresh muscles (see fig .2), may perhaps be due to persistent vasodilatation in the fatigued muscle, because of the metabolites of the preceding muscular activity.

On the basis of previous evidence, curare appears to raise the threshold of the muscle to a-ch (see Rosenblueth and Morison, 1937). The diminished height of contractions in figure 2, after an injection of curare, could thus be accounted for. But there is testimony that the a-ch available in stage *4* is less than in the fresh state (Rosenblueth, Lissák and Lanari, 1939), and therefore it might be supposed that curare would be especially effective in that stage. As shown in figures 2 and 3, however, the drug

has a deeper and more persistent depressive action on the fresh neuromuscular junction than on that which has been subjected to prolonged stimulation until it is well fatigued.

Feng *et al.* (1938) have demonstrated that curare in a small dose abolishes the typical increment of single contractions that follows a brief tetanus, whereas after a larger dose, sufficient to produce a nearly complete synaptic block, the increment reappears. These results were obtained when the neuromuscular junction had not been much stimulated. In our experiments, when the dose of curare was such as to permit a post-tetanic decurarization or increment on the fresh side, the increment did not appear on the side in stage *4* (see figs. 2 and 3). But the muscle in stage *4* was already performing much better than the relatively fresh one. Might it not be possible that the prolonged tetanic stimulation, required to develop stage *4*, resulted in a more or less persistent condition resembling that produced acutely and for a short time after a brief tetanus, i.e., the evoking of some adjuvant agent which renders more effective the a-ch liberated by nerve impulses. This suggestion would imply an agent not readily removed or destroyed. It would imply also that the agent is so prominently present, after the prolonged stimulation leading to stage *4*, that for a considerable period it could not be increased by brief tetani. Thus the greater resistance to curare of the neuromuscular synpase in stage *4*, and the lack of a post-tetanic increment in that stage, could be imagined.

A perplexity arises, however, when this concept is carried over into stage *5*. In that stage the neuromuscular junction is more sensitive to curare than that in stage *4* (see fig. 4), and yet it has been stimulated much longer than the junction in stage *4*. One way out of the difficulty is to suppose that by the time stage *5* is reached the accessory agent has been exhausted or in some manner rendered ineffective. Then the failure of the increment in stage *4* could result from a maximal presence of the agent, as explained above, and the failure in stage *5* could result from its absence or ineffectiveness. The greater sensitiveness to curare in stage *5* would be consistent with this idea.

The post-tetanic increment in stages 4 and 5. Rosenblueth and Morison (1937) suggested that the post-tetanic increment of the responses to single nerve volleys is due to a diffusion of potassium from the muscle during the period of tetanic stimulation. This suggestion was supported by observations of Brown and Euler (1938) and of Feng *et al.* (1938). Feng and his collaborators have shown that an intra-arterial injection of a small amount of potassium chloride has all the effects of a tetanic stimulation, and, furthermore, that these effects are modified by eserine and curare just as are the post-tetanic effects. Feng argues, therefore, that the release of potassium at the neuromuscular synapse in an unusual abundance by rapidly repeated shocks would account for all the post-tetanic phenom-

ena. As remarked above, Feng's observations were made on relatively fresh neuromuscular synapses. Admittedly the duration of the increment of responses after a tetanus is too long to be explained by continued action of a-ch—its existence is ephemeral. An accumulation of potassium at the synapse, however, collaborating with a-ch (see Brown and Feldberg, 1936) would furnish the adjuvant agent invoked in the previous section to provide a concept of what might be transpiring in stages *4* and *5*. Whether prolonged stimulation, such as is required to bring forth stage *4*, would result in a more persistent presence of potassium than results from short stimulation, as was surmised, is not at all evident. And whether a still more prolonged stimulation, that evoking stage *5*, would be attended by a great fall in the potassium output, as was surmised, is also not evident. Concerning the possible coöperation of a-ch and potassium at synapses our knowledge is too meager to justify at this time further pursuit of these speculations.

SUMMARY

The muscular response in stages *4* (fatigue) and *5* (recovery while tetanic stimulation is continued) was studied in relation to certain conditioning factors.

Prostigmin has an augmentive action on the muscular response during stage *4* (see fig. 1); a depressive effect is more marked as stage *5* progresses.

Prostigmin advances the onset of stage *5* and accelerates its development (see table 1).

Curare has an early and precipitate depressive influence in stage *4*, but the recovery from the depression is sooner and faster than in fresh muscle (see fig. 2); i.e., the fatigued synapse is more resistant to curare than the fresh one.

Post-tetanic decurarization does not occur in stage *4* as it does in the fresh state of the synapse (see fig. 3).

Wedensky inhibition (induced by maximal stimuli applied 2 per sec.) occurs to a more marked degree and at a faster rate in fresh than in fatigued muscle.

In stage *5* conditions are more like those of the fresh state than of stage *4*; i.e., the muscle in stage *5* is less resistant to curare than in stage *4* (see fig. 4), and Wedensky inhibition (induced as described above) is more prominent in stage *5* than in stage *4*.

The post-tetanic increment of responses to single nerve volleys, which is evident in fresh preparations, disappears in stage *4*, if the test is made immediately after the fatiguing tetanus. A short period of rest, however, allows the phenomenon to reappear. The increment is absent also in stage *5*, and a longer rest is required for its return than in stage *4*. (See fig. 5.)

In the discussion these results are considered in relation to the chemical and electrical theories of neuromuscular transmission.

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